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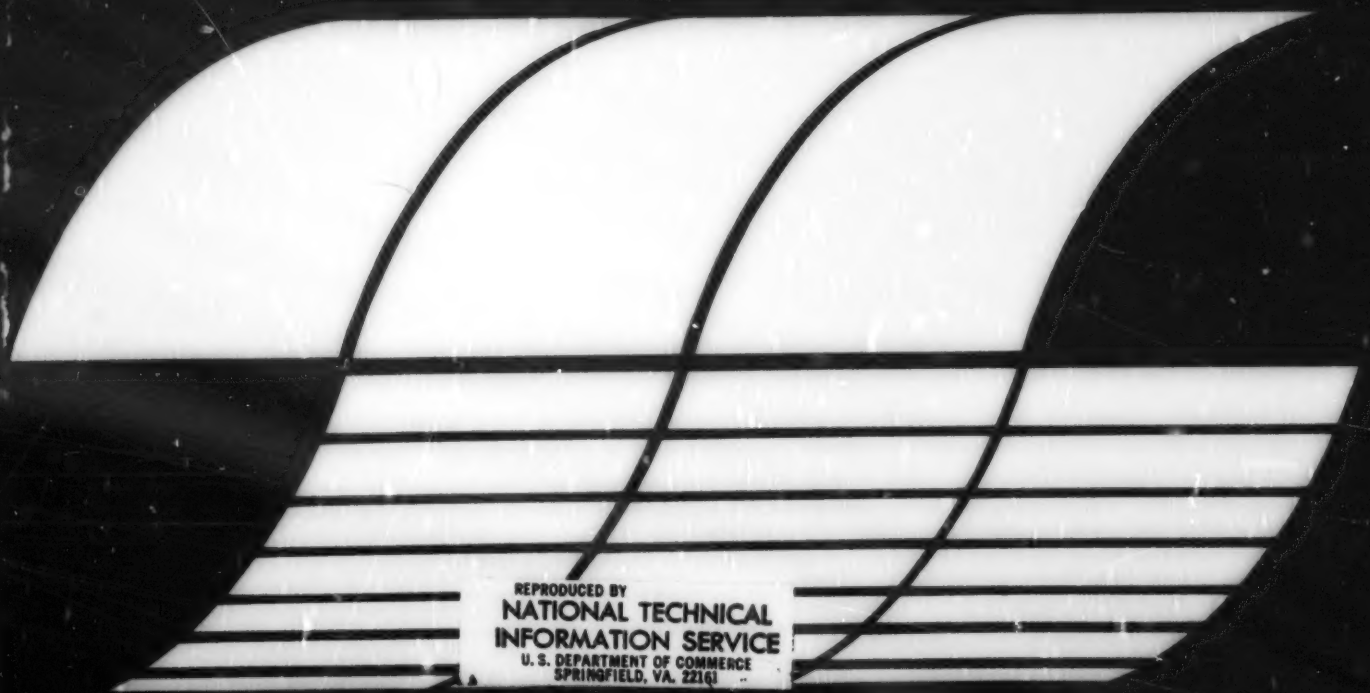

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Petroleum Hydrocarbons In The Northern Puget Sound Area-- A Pilot Design Study

by

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PUGET SOUND ENERGY-RELATED RESEARCH PROJECT
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by

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* Lower case letter (e.g., a) associated with sample number indicates duplicate/replicate aliquots of the composite sample; hyphen (-) denotes "not detected."

ABSTRACT

Hydrocarbon baseline data are needed to assess the potential impact of oil contamination from increased tanker traffic in the Strait of Juan de Fuca. Initial studies were directed to intertidal sediments, mussels and snails from two physically similar areas: Port Angeles, WA and Dungeness Bay, WA. Latest analytical techniques of solvent/slurry extraction, silica gel chromatography, and glass capillary gas chromatography were adapted for analyzing large numbers of samples. The methods apply to alkanes in the C_{14} - C_{32} range, or to aromatics from substituted benzenes through benzpyrenes. Individual hydrocarbons were determined within 20% standard error at the 10 ng/g level.

Lowest hydrocarbon levels were found at Dungeness Bay, a relatively pristine area. The profile of the alkanes suggests that the Dungeness hydrocarbons arise biogenically. Hydrocarbon levels were substantially greater at Port Angeles Harbor, especially adjacent to Peabody Creek. The even distribution of C_{14} - C_{22} n-alkanes from creekside sediment is consistent with known fuel oil seepage upstream. Above C_{22}, the n-alkane pattern appears more biogenic, although the levels are high. Significant divergences between the two areas were found in the phenanthrene fluoranthene, and pyrene levels. Seventeen aromatic hydrocarbons are proposed for baseline monitoring.

FOREWORD

Substantially increased petroleum tanker traffic, pipeline transport, and refining operations are anticipated in the region of Northern Puget Sound and Strait of Juan de Fuca when the Alaska pipeline comes into operation. To assess the potential future environmental impact arising from these activities current hydrocarbon baseline levels must be measured. Under the Puget Sound Energy-Related Project, the NOAA National Analytical Facility (NAF) contracted to undertake a pilot study on the "Design of a Petroleum Hydrocarbon Baseline Investigation for Northern Puget Sound and Strait of Juan de Fuca." This pilot study was supported by U.S. Environmental Protection Agency "pass-through" funds administered by the NOAA Marine Ecosystem Analysis Program. This study was conducted in consultation with representatives from NOAA (National Marine Fisheries Service, Environmental Research Laboratories and Environmental Data Service), Washington State Department of Ecology, University of Washington, U.S. Environmental Protection Agency, and the Canadian Department of the Environment. This report presents the results of the pilot study and offers recommendations for a first year Petroleum Hydrocarbon Baseline Investigation.

INTRODUCTION

The greater Puget Sound region has accommodated the transportation and refining of petroleum and its products for many years without serious difficulties with massive oil spills or smaller scale chronic contamination. Until recently, most of the crude oil requirements of the Pacific Northwest have been supplied to the U.S. refineries in the Puget Sound region by pipeline from Canada. Now, however, this supply has been greatly reduced and probably will cease in the near future. To maintain refinery production, tanker traffic has steadily risen, as has the risk of acute and chronic pollution in the marine environment. This trend can be expected to continue with the opening of the Trans-Alaskan pipeline. Furthermore, it is possible that the greater Puget Sound region could become a petroleum transshipment point servicing other parts of the country.

In a study performed for the Washington State Legislature by the Oceanographic Commission of Washington, it was estimated that refinery capacity could triple and tanker transport of crude oil could increase tenfold by the twenty-first century (1). Major issues thus face the petroleum industry and various levels of governments in this region--issues such as: deciding the ultimate capacity of refining, pipeline and storage facilities; limitations on tanker traffic and location of tanker terminals; and the appropriate response to massive or chronic oil pollution. Decisions resolving issues such as these require a more detailed knowledge of the marine environment of greater Puget Sound than is presently available. Among the various physical, chemical, and biological parameters which need to be better characterized are the hydrocarbon baseline patterns in the environment prior to projected increased petroleum operations.

Knowledge of the present distribution and concentration of hydrocarbons in the environment, especially those found in petroleum, is necessary in order to establish a baseline for measuring the future impact of petroleum pollution. This means that the current conditions need to be well-defined before an effective monitoring program can be designed to determine (a) changes from baseline levels, (b) impacts of pollution, and (c) trends in pollutant concentrations. Furthermore, unnaturally high baseline levels of petroleum components may pinpoint areas exposed to current contamination before the Alaskan oil traffic commences.

Previously, only one systematic study has been conducted in the Puget Sound region to determine the extent of petroleum contamination (2). Currently, Battelle Northwest, under a contract with the U.S. Energy Research and Development Administration (ERDA), is carrying out both biological and chemical studies in the Cherry Point and March Point refinery areas. ERDA has also contracted the University of Washington to undertake chemical studies in these areas. Within NOAA, the Energy Resources Program has a project with the Pacific Marine Environmental Laboratory to analyze the water column, including particulate matter, in the Northern Puget Sound area. The studies recommended herein will supplement and interface with the above projects.

The identification and quantitation of petroleum hydrocarbons in the marine environment is extremely complex. Standardized field and laboratory techniques have been devised only recently. The problem is complicated by biogenic hydrocarbons in the environment, by the complex physical and chemical nature of petroleum, and by uncertainties in analytical and statistical procedures. The validity and utility of baseline data could be questioned unless such problems are resolved. Therefore, it was appropriate to carry out a pilot study of field and laboratory parameters pertinent to the design of a baseline program. This involved workshop panel discussions, field studies and laboratory studies.

A workshop panel was set up to identify important issues and recommend guidelines for the pilot study. The panel consisted of experts in the fields of marine biology, oceanography, analytical chemistry and statistics. Representatives from related programs were included. After the pilot study was largely complete, the data and preliminary conclusions were presented to a second workshop session which discussed aspects of the baseline design.

In the field studies, recommended biota and sediment were sampled at the recommended intertidal sites. The organisms represented two intertidal trophic levels. Methods were established to collect and preserve samples, avoiding contamination. Samples were collected to establish the statistical variability of the procedures.

In the laboratory, various sample extraction methods were assessed for efficiency and precision. Techniques such as adsorption chromatography, microgravimetry, gas chromatography, mass spectrometry, and automation were used to process large numbers of samples. All these studies not only elucidated methods for determining residual hydrocarbons in environmental samples, but they also facilitated the development of specific strategies for the follow-on baseline program.

FIELD STUDIES

Site Selection

The first workshop panel endorsed the recommendation that hydrocarbon residues in samples be compared from two physically similar sites that differ in their known exposure to petroleum contamination. In response to the panel discussions, we chose two areas:

1. Port Angeles Harbor - the possible site of a future supertanker terminal (already exposed to petroleum hydrocarbons), and

2. Dungeness Bay - a wildlife refuge 10 miles east of Port Angeles (presumably relatively uncontaminated).

It was agreed that the sampling should be confined to intertidal sediment and biota (viz., *Mytilus edulis* and *Thais lamellosa*).

Harry Tracy, Washington State Department of Ecology, suggested that the area where Peabody Creek stream empties into Port Angeles harbor might show contamination from a long-standing fuel tank leak. Sampling at a site known to have received chronic exposure to petroleum hydrocarbons would permit a test of the effectiveness of the sampling and analytical procedures when compared with the results from a more "pristine" site.

Three other potential sampling sites in the Port Angeles area were considered: Morse Creek, about 3 miles east of Port Angeles; Francis St. in Port Angeles; and Ediz Hook, also within Port Angeles harbor. However, a field survey of these sites determined that all three sample substrates were found only at the Peabody Creek location. Therefore, the beach at the mouth of Peabody Creek was chosen as the relatively contaminated sampling site.

Three potential locations were also surveyed at Dungeness Bay: Dungeness Spit, with access via Dungeness Beach State Park; Dungeness Beach, at the Three Crabs Restaurant; and Dungeness Beach, 1½ miles west of the restaurant. All three substrates (fine sediment, Mytilus edulis and Thais lamellosa) were found at only the Three Crabs location so it was selected as the relatively uncontaminated sampling site.

During the preliminary survey trip (May 11, 1976) Mytilus specimens were collected in the most accessible locations at Port Angeles and Dungeness: on rocks and pilings of old piers. Analysis showed that the Mytilus attached to pilings at Dungeness contained significant amounts of aromatic hydrocarbons known to be present in creosote. Since this contamination was not directly petroleum related, samples from pilings were henceforth avoided.

Due to the small number of Thais specimens available at Dungeness, another supposedly pristine area, Freshwater Bay, was surveyed. A large population of Thais organisms was found, and specimens of these as well as Mytilus were collected. Examination of the organisms by Tony Roth, University of Washington, revealed that these fauna were of a different species than those at Port Angeles. They were, in fact, Mytilus californianus and Thais emarginata. To avoid potential questions about inter-species variability, it was decided that sampling would be confined to Port Angeles and Dungeness Bay.

Site Description

The outlet of Peabody Creek into Port Angeles harbor is situated at the foot of Lincoln Street (lat. 48°07'14"N, long. 123°25'42"W). A set of pilings in the creek itself forms the reference point from which the sample points were located. The exact sampling points are designated in Figure 1.

At Dungeness Beach, the remaining pilings of an old ferry landing form the reference point for the sampling sites (lat. 48°9'11"N, long. 123°7'11"W) shown on the map in Figure 2.

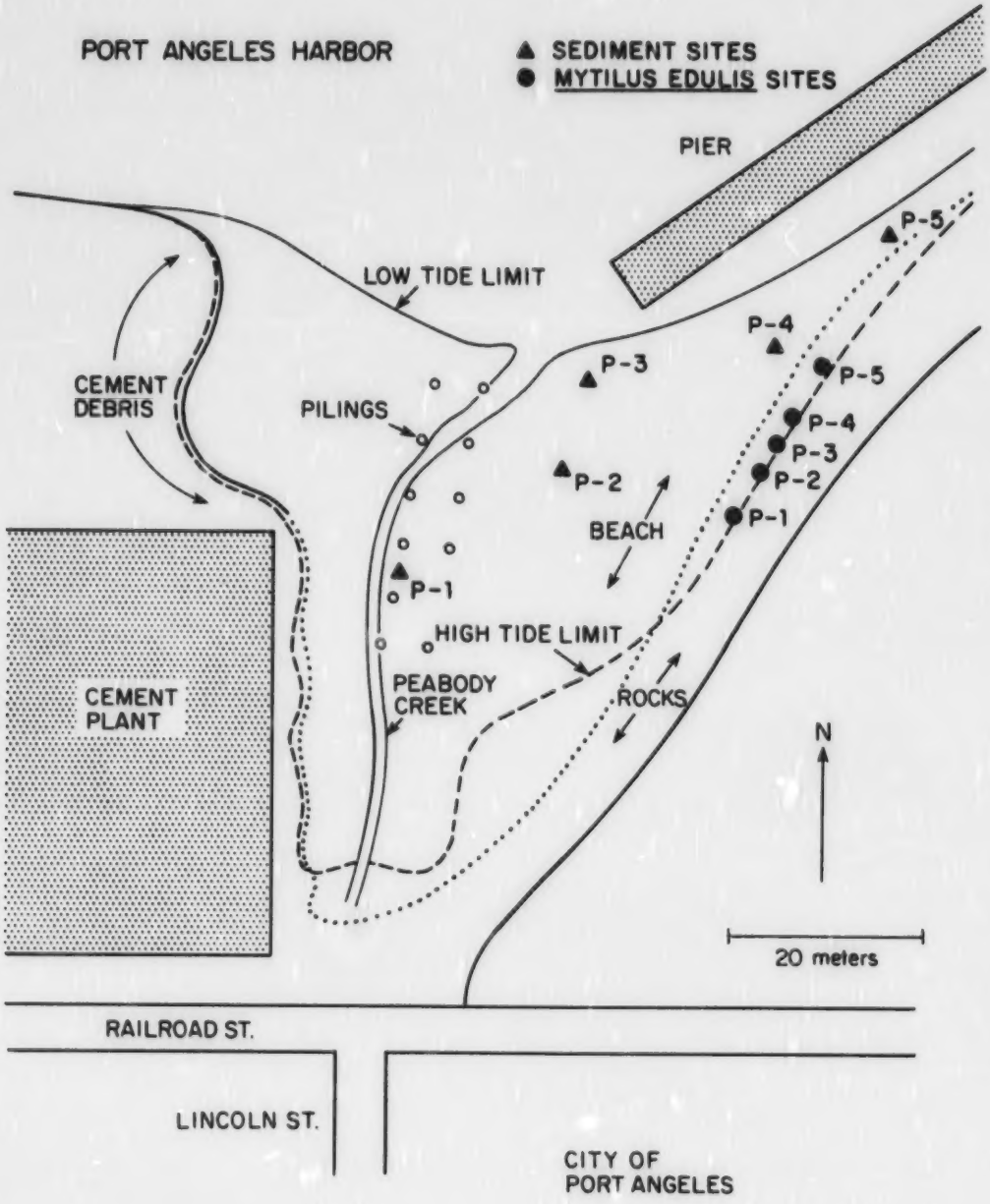

Figure 1. Peabody Creek intertidal sampling area at Port Angeles, Washington.

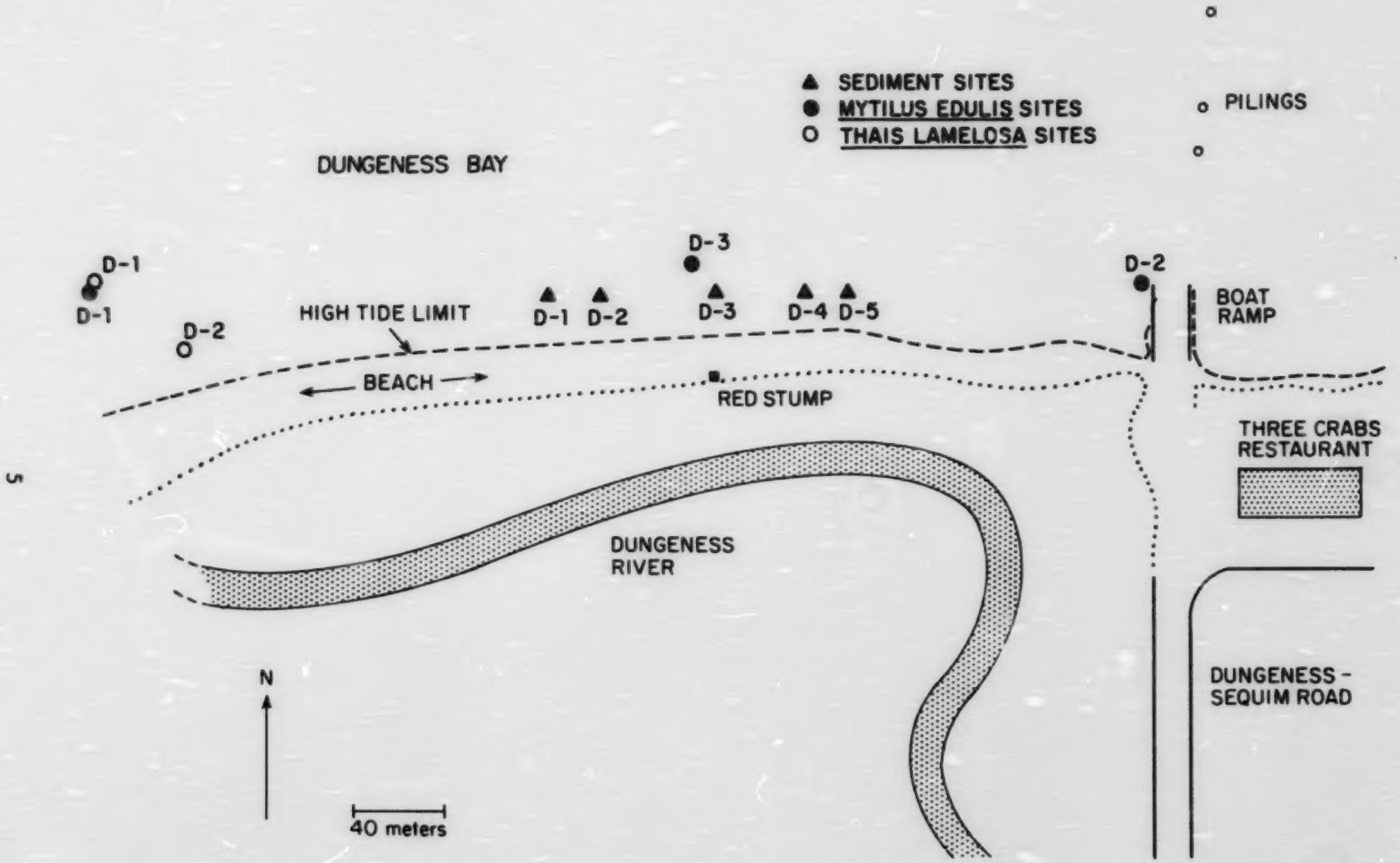

Figure 2. Dungeness Bay intertidal sampling area at Dungeness, Washington.

Sampling Procedures

Composite sediment samples were obtained from multiple cores using 3 cm deep by 7.5 cm diameter tin cans previously cleaned with solvents and 96% sulfuric acid. These incremental samples were taken at the corners of a square meter area and placed in a clean aluminum pail. The samples were composited by thoroughly mixing with a hand trowel. A portion of each composite (an aliquot) was transferred to either clean, wide-mouth glass jars or aluminum foil. At both Port Angeles and Dungeness Bay, the entire sample of one sediment composite was retained to allow multiple replicate analyses.

For each composite sample, ten specimens of Mytilus edulis and of Thais lamellosa were collected by hand and wrapped in foil for transport and storage. Mytilus was relatively abundant at each location and presented no collection problems; however, only enough Thais specimens could be found to make one sample composite at Port Angeles and two sample composites at Dungeness. All samples were stored in a cold chest for transport and placed in a freezer (-20°C) within 6 hours.

LABORATORY STUDIES

Analytical Overview

For more than a decade, petroleum chemists, organic geochemists and chemical oceanographers have been applying modern analytical organic methods to analyze marine sediments and biota for traces of hydrocarbons. These developments are reviewed biennially (odd years) in Analytical Chemistry under "Petroleum" (3). Recently, the need to simplify and harmonize the numerous, lengthy, painstaking, and individualized procedures has led to a series of conferences and studies focusing on these problems. In the workshop of 26 scientists, funded by NOAA in 1972 at the Santa Catalina Marine Biological Laboratory, entitled: "Marine Pollution Monitoring: Strategies for a National Program," Farrington, Giam, Harvey, Parker, and Teal (4) examined the analytical situation for petroleum contamination and recommended specific monitoring procedures. The following year the National Academy of Sciences sponsored a larger workshop (62 scientists) devoted to "Petroleum in the Marine Environment" (5). Chapter 2, "Analytical Methods," contains numerous brief yet explicit descriptions of useful techniques. Most analytical methods for hydrocarbons in the marine environment follow a basic scheme: substrate digestion, where necessary, followed by solvent extraction, then adsorption chromatography for sample cleanup or class separation, and gas chromatography (GC) to determine the hydrocarbon constituents. A somewhat different approach to hydrocarbon baseline analysis was taken by the National Bureau of Standards in a study on the Gulf of Alaska (6). Their procedure employs gas stripping of the volatile compounds which are trapped on Tenax-GC polymer and analyzed by capillary GC. Residual compounds are analyzed, in part, by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The "Pilot Study of the Buccaneer Oil Field" (7) in the Gulf of Mexico follows aspects of several analytical protocols, but mainly that of Farrington et al. (4).

Despite the above efforts (or perhaps because of them), it has since become evident that variations in methodology should be intercompared to determine which options are best for a "standard method." In their report to the Bureau of Land Management (BLM), "Evaluation of Extraction Techniques for Hydrocarbons in Marine Sediments," Rohrback and Reed (8) describe such experiments. Their recommendations closely resemble the current "official" BLM procedures for hydrocarbon baseline analyses (9).

In our efforts to evaluate and recommend analytical procedures for the hydrocarbon baseline studies described in this report, the Rohrback and Reed report (8) provided valuable insight. Specifically, their experiments showed Soxhlet extraction to be only slightly more efficient (4%) than simple solvent extraction with agitation. Furthermore, the Soxhlet extraction required time-consuming, freeze-drying of the sediment for best results. In contrast, Warner (10) proposed a simpler procedure in which an aqueous slurry of sediment was extracted when tumbled in contact with diethyl ether. Admittedly, Soxhlet extraction is important with difficult-to-extract, compacted, fossil sediment (i.e., rocks), but it offers no evident advantage here with unconsolidated sediments.

After sample extraction, both the Warner procedure (10) and the BLM procedure (9) recommend adsorption chromatography for sample extract cleanup and hydrocarbon group classification, followed by GC determination of hydrocarbon constituents. From time to time, shortcuts to these procedures are proposed for assessment of petroleum pollution such as gross analysis of the extracts by infrared, UV, or fluorescence spectrometry. However, Gordon, Keizer, and Dale (11) point out that if the chemical composition of the substrate is unknown and free to vary, as is usually the case, quantitative results have no meaning because the reference calibration cannot be defined. This problem is not serious with capillary (high-resolution) gas chromatography because of its capability to separate complex hydrocarbon mixtures into hundreds of constituents and measure many of them at 10^{-9} g levels or lower. These separated compounds may be provisionally identified by comparing their GC retention times with that of the corresponding known standard. This standard also serves to calibrate the quantitative response factor. Confirmation of identity may be obtained by an equally sensitive and useful technique, gas chromatography/mass spectrometry (GC/MS). The popularity of GC for hydrocarbon analysis can be further attributed to its comparatively low cost and broad analytical range which covers hydrocarbons from C_1 to over C_{40}. Although GC/MS is more expensive, it is needed to identify unknown compounds and to confirm provisional GC identifications.

A more recent development, high-performance liquid chromatography (HPLC) with UV absorbance or fluorescence detection, is becoming increasingly useful for analysis of aromatic hydrocarbons in marine substrates (6, 10), especially with compounds which are not sufficiently heat-stable or volatile for GC. One promising HPLC application for aromatic hydrocarbons is a group-analysis approach which totals the compounds having a like number of benzene rings (one, two, etc.).

Specific Laboratory Studies

We have evaluated the latest analytical procedures, techniques, and equipment to determine the best means of obtaining convenient, reliable, and useful quantitative measurements of petroleum hydrocarbons in marine sediments and tissues. Historically, this has not been an easy task, especially for large numbers of environmental samples. Rohrback and Reed (8) provided valuable information on the extraction of sediments and other analytical techniques. We made detailed comparisons of the techniques of Warner (10), BLM (9), NBS (6), and Rohrback and Reed (8). After careful consideration, Warner's procedures (10) were adopted as the framework for further development. With several important modifications, they constitute the procedures currently recommended by this laboratory (Appendix A).

In our procedures (Fig. 3), acidified sediment or alkali-digested, homogenized tissue is extracted with ether and chromatographed on silica gel. The latter separates the ether soluble extract into two fractions: the saturated hydrocarbons and the unsaturated hydrocarbons. The fractions are concentrated separately; those from sediment are desulfurized with activated copper prior to further analysis. An aliquot of each fraction is weighed on a microbalance, and the weight is compared to the dry weight of the sample. Each fraction is then analyzed by automated gas chromatography (GC), using high resolution glass capillary columns for quantitation of specific compounds. The identity of all hydrocarbons reported is periodically verified by gas chromatography/mass spectrometry (GC/MS). Details of the analytical procedures appear in Appendix A.

Our method differs from Warner's (10) in the following respects:

1. Tissue digestion. Tissue samples of marine organisms were digested in alkali overnight at 30°C in Teflon-lined, screw-capped centrifuge tubes. In the Warner procedure (10) similar digestion for 2 hours at 90°C showed frequent losses of moderately volatile hydrocarbons (e.g., substituted naphthalenes), due to imperfectly sealing caps.

2. Silica gel. A coarser grade (100-200 mesh) of silica gel (MCB SX0144-06) gave satisfactory class separation of the saturated and unsaturated hydrocarbons. Warner's procedure (10) required pneumatic pressurization with nominal 200 mesh and finer silica gel.

3. GC columns. High-resolution glass capillary columns were used instead of packed columns. The capillaries gave much better hydrocarbon separations than packed columns, and they also allowed the aromatic hydrocarbon class to be analysed as a single GC sample rather than as two samples.

Depending on the size and nature of the sample, selected individual hydrocarbons can be detected and measured from parts-per-million (ppm) down to parts-per-billion (ppb) levels, based on dry weight. For tissue samples the

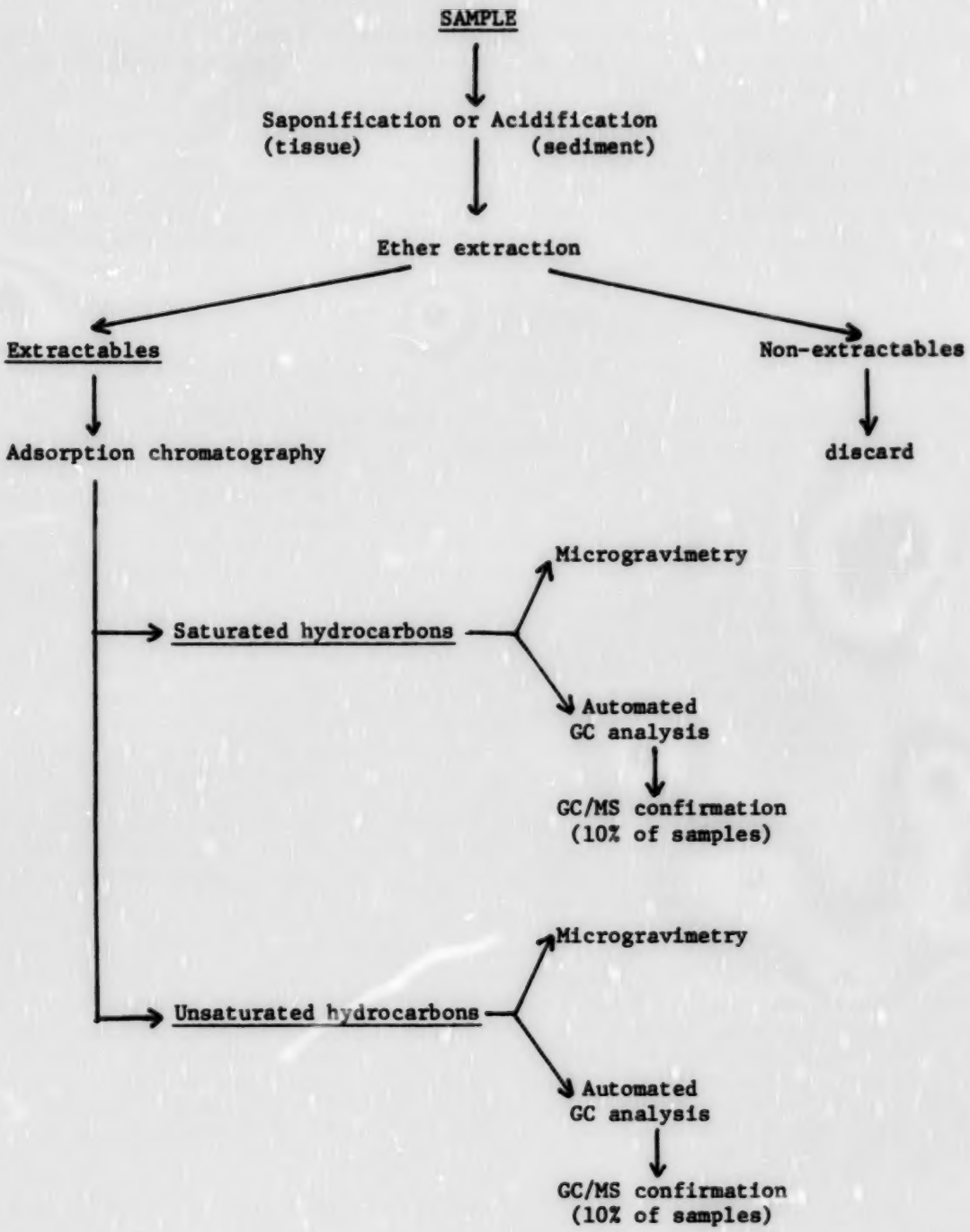

Figure 3. Schematic of tissue and sediment analysis.

sensitivity is currently about 20 ppb, for sediment it is about 1 ppb. When available, the GC manufacturer's glass capillary and pre-column assembly should improve this sensitivity 5- to 100-fold.

Tissue Digestion Studies. Tissue samples spiked with moderately volatile hydrocarbons were digested in alkali (NaOH) at 90°C for 2 hours in 40 ml centrifuge tubes sealed with Teflon-lined screw caps according to the Warner procedure (10). About 90% of the added compounds were lost in these digestions probably due to poor sealing of the tube caps and the elevated temperature. Alternatives that were tested include:

1. Enzyme digestion (Papain), 25°C;
2. Combination of enzyme and alkali (NaOH) digestion, 25°C; and
3. Alkali only, varying the NaOH concentration and the digestion temperatures and times.

Enzyme digestion with 0.4-1% purified papain was unacceptable because a stable emulsion formed during solvent extraction of the digestion mixture. Among several other experiments, we found that tissue was satisfactorily digested in 4N NaOH at 30°C in 16-18 hours (overnight). This gave a 90% recovery of spiked hydrocarbons (C_{14} and up).

Sediment Extraction Studies. Several techniques for sediment extraction were considered including: (a) exhaustive Soxhlet extraction, (b) agitation on a shaker table, (c) refluxing, and (d) rolling on a ball-mill tumbler. A study comparing the first three of these methods was made by Rohrback and Reed (8) for the BLM. They favored Soxhlet extraction; however, Soxhlet extraction for 50 hr was only 4% more efficient than shaker table extraction for 20 hr. Refluxing was the least preferred of these methods. A fourth method, developed by Warner (10), consisted of extracting an aqueous slurry of the diethyl ether by rolling the mixture in a sealed bottle on a ball-mill tumbler. This method, similar to the shaker table technique, was adopted because it is both efficient and convenient.

Extraction Solvent Studies. Diethyl ether with 2% ethanol preservative is not acceptable for this procedure. Up to 0.5 ml of ethanol remains in the extract and upon concentration ethanol separates as a second phase. This ethanolic phase may cause bumping and loss of sample. Furthermore, the alcohol may deactivate the silica gel column and nullify the separation of saturated and unsaturated hydrocarbons. Freshly-opened, unpreserved ethyl ether must be used to avert these problems. The level of the diethyl peroxide should be monitored with peroxide test paper. If the peroxide concentration exceeds 15 ppm, the ether should be purified or discarded.

Extract Concentrating Studies. Several solvent concentrating techniques were evaluated including: (a) evaporation under a stream of dry nitrogen, (b) evaporation on a Kontes tube heater, and (c) a combination of the two. Solvent evaporation under nitrogen alone was too slow to be useful. Evaporation with the tube heater was faster when combined with the nitrogen stream

technique. Either choice gave comparable hydrocarbon recoveries. Solvent boiling techniques employing heat required an ebullator (boiling tube) to prevent bumping and resultant loss of sample. Ultimately, an aluminum-foil shroud used around the Kontes heating apparatus and tubes facilitated evaporation by heat. This obviated the need for a nitrogen stream.

Eliminating Gels. Some tissues extracted with diethyl ether will form a gel upon solvent evaporation. This highly viscous material can plug an adsorption chromatography column, preventing solvent flow. However, the sample gel can be dissolved in methylene chloride and the causative agents can be removed from the extract solution by filtering the solution through a bed of chromatographic silica gel prewashed with methylene chloride. The methylene chloride solvent is then displaced by hexane for adsorption chromatography.

Adsorption Chromatography Studies. We modifed the silica gel chromatography technique to attain the highest possible flow rate at ambient pressure, while resolving (separating) the saturated hydrocarbons from the unsaturated hydrocarbons. The MCB silica gel (nominal 200 mesh and finer) normally used gave very slow flow rates (<0.5 ml/min) and were easily plugged by moderately viscous extracts. Faster flow rates were obtained using less silica gel but the class separation was incomplete. Several chromatographic adsorbents (silica gels, aluminas, magnesium silicate) in the 100-200 mesh range were evaluated and found to differ widely in their hydrocarbon resolving characteristics. MCB SX0144-06 (100-200 mesh) silica gel gave a desirable flow rate, and it completely resolved the saturated hydrocarbons from the alkyl-substituted, aromatic hydrocarbons.

Detailed column packing techniques were developed for preparing uniform silica gel columns (see Appendix A). Solvent ratios and fraction volumes were evaluated to optimize the separation of saturated hydrocarbons from polyunsaturated and aromatic hydrocarbons to give two fractions of minimum volume.

Desulfurization Studies. GC/MS analysis of sediment extracts showed sulfur (S_8) in virtually all instances. Although S_8 does not interfere with GC analysis, it does interfere with GC/MS analysis and microgravimetry. Sulfur is removed by contact with activated copper (see Appendix A). Initially, fine granular copper was cleaned with concentrated HCl, then washed with acetone, oven dried at 80°C and stored under petroleum ether. However, when used in the procedure, GC/MS analyses proved that S_8 removal was incomplete. Oven drying apparently reverses activation of the copper because when this step was omitted, the copper effectively desulfurized sediment extracts. This procedure will have to be reinvestigated for analyses of organosulfur compounds, in which case sulfur levels will have to be reduced effectively without disturbing the organosulfur compounds.

Microgravimetric Studies. Microgram weighing procedures were evaluated using a Cahn microbalance. When aliquots of solutions of pure compounds, such as pentadecane, were air-dried and weighed, the residues indicated that about 10% was recovered. Extracts of environmental samples in solvent generally contain non-volatile material which acts as a "keeper" to minimize losses of

moderately volatile hydrocarbons. In an analogous weighing experiment microgram amounts of a light machine oil dissolved in solvent lost only about 10% due to evaporation.

Solvent Displacement for GC Analysis. The solvents used in silica gel chromatography were displaced by carbon disulfide to minimize their interference with the GC detector. In the first experiments, the fractions from silica gel chromatography were evaporated in the heater block to 0.5 ml, then placed in uncapped GC vials, and allowed to evaporate to dryness at room temperature. The residues were taken up in 0.5 ml carbon disulfide (CS_2) and analyzed by GC. Analysis showed that only about 10% of pentadecane and naphthalene was recovered from spiked samples. Subsequently, the expected analytical recovery efficiency (70-95%) was attained by avoiding complete evaporation. In this procedure, the petroleum ether and/or methylene chloride solvents were displaced with CS_2 by adding 1 ml CS_2 to the 0.5 ml chromatographic concentrate. Then the mixture was reconcentrated to 0.5 ml in the heater block. Conveniently, an internal standard, such as hexamethylbenzene, can be added in the 1 ml CS_2.

Gas Chromatography. The tissue and sediment extracts are extremely complex organic mixtures. A GC system capable of the highest possible resolution is needed to obtain useful analyses from such samples. Since packed columns are limited in separation capability compared with capillary GC columns, capillaries were chosen for this study. Many instrumental modifications of both the GC and GC/MS systems were necessary to achieve optimum configurations for capillary column operation (see illustrated details in Appendix A).

ANALYTICAL RESULTS

Over 60 composite marine intertidal samples of sediment, mussel (Mytilus) and snail (Thais), were extracted for residual hydrocarbons and analyzed according to the recommended procedure. The saturated and unsaturated hydrocarbon classes separated by adsorption chromatography were determined by microgravimetry. Selected individual hydrocarbon compounds were determined by GC. Each composite was analyzed in replicate (duplicate or more) as denoted by the sample code (P-1a, P-1b, etc.). The results of these analyses appear in Tables 1-9.

In many instances the agreement of the amounts of individual compounds (Tables 2,3,5,6,8, and 9) from duplicate analyses is excellent. Other instances show varied comparisons, i.e., one compound may be high in sample a, low in sample b, whereas the reverse is true for a different compound in the paired samples. Occasionally the difference is biased one way throughout the range of compounds.

Table 10 shows the GC reproducibility of a single extracted sample injected in replicate (5 times). Although further improvement in these data can be virtually assured at this time, it can be seen that the GC precision shown in Table 10 is good-to-excellent except for the n-C_{30} and n-C_{31} alkanes.

As far as the overall analytical procedure is concerned, the relative standard deviation (or coefficient of variation) at the 95% confidence level averaged less than 20% for individual hydrocarbon compounds at the practical limit of sensitivity. For 100 g sediment samples (wet), the practical sensitivity limit is 1 ng/g dry sediment or 1 part per billion (1 ppb). For 10 g of wet mussel tissue it is about 20 ppb (dry wt). Anticipated improvements in the GC sample introduction apparatus should improve these sensitivities from 5- to 100-fold.

The objective of these analyses was to determine the abundance and variation of residual hydrocarbons in marine intertidal sediments and biota. Comparative differences between these hydrocarbon levels from a relatively unpolluted and a polluted site were investigated. Table 1 shows the comparative residual levels of the hydrocarbon classes found in sediments at Port Angeles harbor and Dungeness Bay. The supposed greater exposure to petroleum related hydrocarbon pollution at Port Angeles is indicated in the higher levels of saturated hydrocarbon (chromatography fraction 1) found in Port Angeles sediments (samples P-1 to P-5) compared to those found in Dungeness (D-1 to D-5). These differences are also reflected in the comparative levels of the selected individual saturated hydrocarbons (alkanes) shown in Table 2. The total unsaturated hydrocarbons (chromatography fraction 2) at Port Angeles and Dungeness (Table 1) mirror this situation, although not to the same degree of difference. This lesser difference is also borne out by the comparative levels of selected predominant arenes (aromatic hydrocarbons) in the unsaturated fraction (see Table 3).

The evidence of the Port Angeles microgravimetric data (Table 1) strongly suggests that these hydrocarbon class levels are related to known hydrocarbon contamination. Specifically, Peabody Creek, which empties into Port Angeles

Table 1. Microgravimetric analysis of hydrocarbons extracted from intertidal sediment, Port Angeles harbor (P) and Dungeness Bay (D).

Sample	Silica Gel Chromatography Fraction (µg/g dry sediment) 1. (saturated)	2. (unsaturated)
P-1 a	950	360
b	770	350
P-2 a	76	95
b	78	94
P-3 a	1000	430
b	1100	400
P-4 a	170	160
b	130	190
P-5 a	110	360
b	100	84
c	100	180
d	110	180
e	73	140
D-1 a	2.5	34
b	2.7	37
D-2 a	5.5	29
b	3.7	27
D-3 a	2.8	23
b	1.5	24
c	2.9	33
d	1.8	29
D-4 a	2.7	29
b	3.2	17
D-5 a	3.9	36
b	4.2	33

Table 2. Alkanes extracted from intertidal sediment, Port Angeles harbor (P) and Dungeness Bay (B).

Alkane* concentration, ng/g of dry sediment

Sample		C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	Pristane	Phytane	Σodd-C	Σeven-C
P-1	a	290	370	300	420	290	370	230	220	180	450	270	1700	630	1700	320	870	94	1100	400	290	7200	7200
	b	210	380	300	410	300	370	250	250	210	480	290	1400	670	1800	350	750	240	1600	370	280	7140	2820
P-2	a	<2	19	15	25	17	13	18	28	22	250	9.9	1200	120	2300	270	1400	150	870	47	24	6205	602
	b	8.6	20	15	24	16	10	18	30	23	150	9.5	1000	230	1400	150	1400	150	820	47	25	5554	520
P-3	a	170	200	180	240	130	34	110	32	74	170	120	630	210	930	290	470	88	430	240	94	3136	1372
	b	160	190	170	240	120	32	110	32	60	170	92	570	170	870	240	450	88	360	230	99	2914	1210
P-4	a	30	43	38	64	33	<2	28	22	16	14	3.4	140	78	250	160	150	<2	160	77	43	843	386
	b	25	35	32	50	31	<2	26	2	2	21	6.6	130	69	240	180	170	<2	180	74	42	826	370
P-5	a	22	35	35	50	35	30	28	33	28	47	35	130	50	210	40	120	<2	110	74	42	765	277
	b	12	17	27	42	25	55	26	22	28	76	43	140	46	190	20	66	<2	130	58	37	738	230
D-1	a	9.1	42	11	26	11	19	11	15	8.0	33	13	130	13	160	56	110	4.8	44	33	12	580	140
	b	9.0	45	11	30	12	20	10	16	9.8	34	14	120	13	160	19	100	2.5	41	36	15	570	100
D-2	a	12	87	18	31	16	27	15	21	12	42	15	110	13	190	48	150	13	62	45	11	720	160
	b	10	73	15	29	14	26	12	21	13	43	17	140	26	220	23	70	14	88	41	10	710	140
D-3	a	4.8	31	8.5	13	11	17	30[c]	10	10	42	23	130	25	230	100[c]	180	12	110	16	5.2	763	234
	b	5.7	31	7.7	11	6.5	13	5.7	11	6.5	30	16	110	18	160	25	120	8.8	66	16	4.5	552	94
D-4	a	12	71	16	32	15	31	16	26	18	76	25	220	8.9	350	21	68	1.9	82	59	16	960	130
	b	11	72	17	34	16	36	19	33	32	100	50	300	21	450	40	71	2.6	57	62	16	1150	210
D-5	a	4.3	20	6.5	15	6.5	11	7.5	12	9.2	36	14	110	8.4	210	10	66	8.3	55	23	5.1	540	80
	b	4.1	23	6.6	13	7.0	10	8.2	13	9.8	35	16	120	17	220	20	49	3.9	77	22	5.5	560	90

* normal alkane denoted where chain length given as C_{14}, C_{15}, etc.

c denotes contaminated peak

Table 3. Selected aromatic hydrocarbons extracted from intertidal sediment, Port Angeles harbor (P) and Dungeness Bay (D).

Sample		Concentration, ng/g dry sediment		
		Phenanthrene	Fluoranthene	Pyrene
P-1	a	320	730	450
	b	240	1000	480
P-2	a	43	120	110
	b	50	110	100
P-3	a	190	660	340
	b	230	1100	510
P-4	a	320	600	390
	b	110	370	300
P-5	a	37	180	110
	b	18	190	190
	c	30	180	200
	d	36	150	150
	e	87	230	260
D-1	a	11	17	13
	b	6.7	18	17
D-2	a	11	24	24
	b	5.4	13	13
D-3	a	1.2	10	13
	b	3.8	25	13
	c	3.3	7.5	13
	d	3.1	8.0	15
D-4	a	11	22	11
	b	-	-	-
D-5	a	-	6.4	6.4
	b	3.9	7.0	7.0

harbor at the sampling site (Fig. 1), was reported to have been previously contaminated by a long-standing fuel tank leak. Table 1 shows that sediment samples taken adjacent to the Peabody Creek stream bed (samples P-1 and P-3) contained up to 10-fold higher levels of saturated hydrocarbon residues (chromatography fraction 1) than samples on the same beach farther from the stream bed (samples P-2, P-4, and P-5). The unsaturated hydrocarbon fraction (chromatography fraction 2), containing the arenes also reflected this trend, though to a lesser degree. The data in Table 3 suggest that the three selected arenes (phenanthrene, fluoranthene, and pyrene) may be possible petrogenic indicators. Again, the highest values were adjacent to the Peabody Creek, Port Angeles, and the lowest values were at Dungeness. In addition to data on levels of hydrogen classes and specific compounds (Tables 1-9), the actual gas chromatograms (GC charts) can aid in diagnosing a pristine vs. contaminated situation. For example, the n-alkanes from sediment samples show an odd-carbon predominance over even-carbon in the range of n-C_{26} to C_{31} for both Port Angeles (Fig. 4a) and Dungeness (Fig. 4b). This odd-carbon predominance is believed to be due to terrestrial biogenic input in both cases; however, in the region around n-C_{15} the comparison changes. Although the n-alkanes from Dungeness sediments display a marked odd-carbon predominance over even, this situation is not reflected in the n-alkanes of Port Angeles sediment. Since it is known that diesel oil has contaminated Peabody Creek for a long time at Port Angeles, it is not surprising that the C_{14}-C_{20} n-alkanes show less alternation. The size of the extensive hump of unresolved compounds above the usual GC baseline rise in Figure 3a also suggests petrogenic contamination. Finally, Fig. 4b is much less complex than Fig. 4a. Thus, significant qualitative information can be gained by visual inspection of the chromatograms.

Chromatograms of the arenes from sediment (Fig. 5) are also significantly simpler from Dungeness (Fig. 5b) than from Peabody Creek, Port Angeles (Fig. 5a). Unfortunately, visual differences are not as clear with Mytilus or Thais arene gas chromatograms; biogenic hydrocarbons complicate the GC picture such that it may be preferable to rely on tabular abundancy data of selected compounds (Tables 5,6,8, and 9).

GC/MS was used to identify or verify the identity of all hydrocarbon compounds listed in the tables. Table 11 lists the unsaturated compounds identified from sediments sampled adjacent to Peabody Creek, Port Angeles. Most are arenes commonly occurring in petroleum and its products. Pinene, a natural alkene, and dichorobenzene, a petrochemical, were also identified.

Composite intertidal samples of mussels (Mytilus edulis) could not be obtained immediately adjacent to the Peabody Creek bed, therefore, where they were found could be presumed to reflect more the general harbor pollution at Port Angeles than the specific fuel tank contamination. Saturated hydrocarbon levels (chromatography fraction 1) at Port Angeles (Table 4) predominate over those at Dungeness in all but one instance (Sample D-2). On the other hand, microgravimetric data on the unsaturated hydrocarbons (chromatography fraction 2) from mussels seem to bear little relationship to hydrocarbon contamination due to the predominance of biogenic olefins (10) in this fraction.

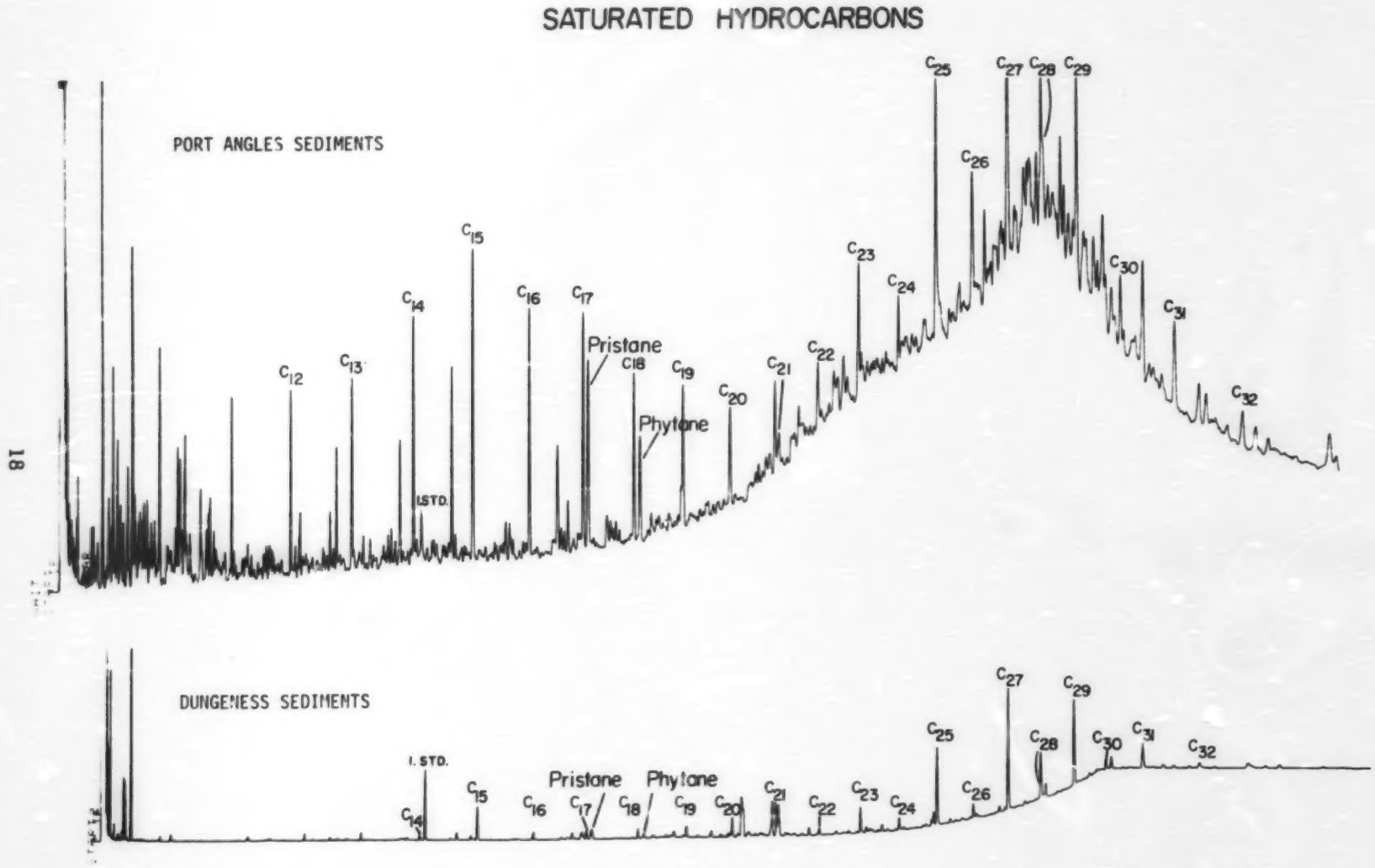

Figure 4. Gas chromatograms of saturated hydrocarbons extracted from (a) Port Angeles sediments and (b) Dungeness sediments.

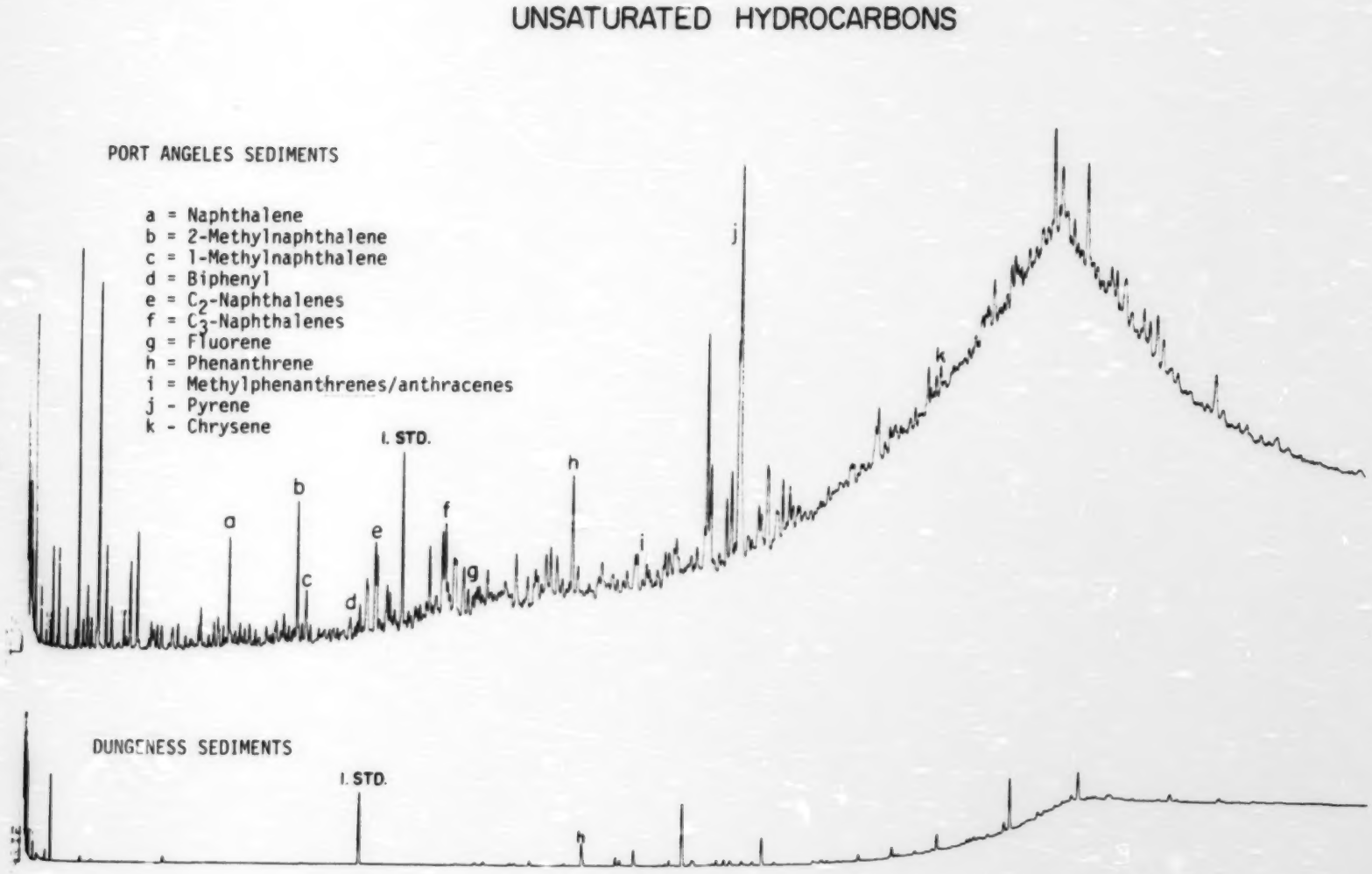

Figure 5. Gas chromatograms of unsaturated hydrocarbons from (a) Port Angeles sediments and (b) Dungeness sediments.

Table 4. Microgravimetric analysis of hydrocarbons extracted from Mytilus edulis tissue, Port Angeles harbor (P) and Dungeness Bay (D).

Sample		Silica Gel Chromatography Fraction (µg/g dry tissue) 1. (saturated)	2. (unsaturated)
P-1	a	350	1200
	b	310	710
P-2	a	350	810
	b	220	420
	c	460	550
	d	300	780
	e	350	880
P-3	a	450	410
	b	380	490
P-4	a	260	360
	b	250	700
P-5	a	340	540
	b	370	450
D-1	a	31	720
D-2	a	123	340
	b	340	630
D-3	a	21	1000
	b	51	560
	c	58	690
	d	35	670
	e	63	620

Many of the individual alkanes (viz., n-C_{14}, n-C_{18}, n-C_{19}, n-C_{20}, n-C_{22}, n-C_{24}, n-C_{26}, pristane and phytane) extracted from mussels are much more abundant in the Port Angeles samples than in the Dungeness samples (Table 5). This may reflect petrogenic hydrocarbon contamination. In contrast, most levels of the prominent, odd-numbered n-alkanes do not differ sufficiently between Port Angeles and Dungeness mussels to indicate petroleum contamination. Under the GC analysis conditions, the n-C_{28} alkane in mussels co-chromatographs with a major biogenic (terpanoid) hydrocarbon and, therefore, cannot be used as a petrogenic indicator. The data also show n-C_{30} to be of little aid. The comparative levels of the selected arenes in the mussels from the two areas (Table 6) indicate that these compounds could be significant in determining petrogenic contamination.

Among snails studied from both areas, the microgravimetric data on the hydrocarbon classes (Table 7) show no significant differences. Except for pristane and phytane in Table 8, the same is true for the alkanes. As with mussels, the abundance of selected arenes in snails (Table 9) reflect the assumed contamination at Port Angeles when compared to Dungeness. However, Thais lamellosa is not promising for baseline studies because its abundance at a given place and time is unpredictable.

In this particular study, it was convenient to use three predominant arenes in the unsaturated fraction (phenanthrene, fluoranthene, and pyrene) to indicate the degree of possible petrogenic contamination. For the follow-on baseline study, GC conditions should be optimized to cover the expanded list of arenes in Table 12. These compounds were selected because (a) they are found in crude and refined petroleum, (b) they are recovered efficiently by our procedures, and (c) they cover a wide range of arenes from 1-5 rings, yet can be determined in a single gas chromatogram. The n- and i-propylbenzenes are about 60% recoverable, naphthalene about 70%. Because of their lower volatilities, the rest of the arenes in Table 12 are over 80% recoverable in sample workup.

The benzenes and naphthalenes are the most abundant arenes in crude oil, as well as the most water-soluble, volatile, and acutely toxic. Hence, the benzenes and naphthalenes deserve a prominent place in a baseline survey. Unfortunately, beyond the C_2 substituted members, the GC pattern becomes difficult to manage with all the possible chemical isomers, thus the list is limited to members which are prominent in pollution and well separated by GC. The polycyclic arenes, though less abundant and less water-soluble, are important for their relationship to possible chronic biological effects. More information is needed on their accumulation in the environment. The polycyclics include: fluorene, phenanthrene and anthracene, methyl phenanthrenes and anthracenes (3-ring arenes); fluoranthene, pyrene, chrysene, benzanthracene (4-ring arenes); and the benzpyrenes, and perylene (5-ring arenes).

Table 5. Alkanes extracted from Mytilis edulis, Port Angeles harbor (P) and Dungeness Bay (D).

Alkane* concentration, ng/g dry tissue

Sample		C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	Pristane	Phytane	Σodd-C	Σeven-C
P-1	a	360	150	170	33	100	-	170	-	-	100	<20	60	<20	130	370	230	70	-	1000	350	703	1240
	b	150	180	140	300	33	-	160	-	-	65	30	98	65	130	390	200	65	30	1000	280	1003	1033
P-2	a	190	370	92	120	61	61	180	310	120	220	120	340	250	310	64	310	-	-	250	150	2041	1077
	b	170	330	59	59	30	30	170	270	120	130	130	120	120	59	30	-	-	-	810	280	998	829
	c	340	330	92	150	61	210	340	700	180	190	150	280	280	120	61	-	-	-	640	430	1980	1504
	d	190	430	540	340	280	170	280	440	240	280	220	400	280	220	93	130	-	-	1000	420	2410	2123
	e	450	220	59	400	30	-	420	440	59	180	89	410	240	270	510	180	-	-	1200	450	2100	1857
P-3	a	140	140	68	170	27	-	-	-	34	68	-	190	68	-	-	-	-	-	1700	510	568	337
	b	150	130	65	130	65	-	-	-	48	65	33	100	65	33	65	-	33	33	1700	590	491	491
P-4	a	190	180	220	330	-	-	100	-	-	66	33	200	130	130	260	230	33	-	830	260	1136	966
	b	290	130	-	210	-	-	-	-	-	-	-	130	100	100	510	300	-	-	130	170	870	900
P-5	a	150	150	200	230	-	-	100	-	-	66	-	100	-	130	700	-	-	-	660	230	676	1150
	b	220	170	130	290	-	-	-	-	-	<20	-	160	-	160	250	190	-	-	870	260	970	600
D-1	a	-	110	-	76	-	-	-	-	-	-	-	-	-	-	-	-	-	-	390	-	186	0
D-2	a	-	66	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	166	0
	b	-	100	-	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	240	0
D-3	a	-	200	-	100	-	-	-	100	-	33	-	-	-	170	630	100	100	-	-	-	703	730
	b	33	210	-	180	-	-	-	620	-	-	-	65	-	10	590	100	65	-	-	-	1275	688
	c	35	210	-	70	-	-	-	70	-	-	-	100	-	240	920	100	35	-	-	-	790	990
	d	33	210	33	120	-	-	-	320	-	33	-	130	<20	290	1600	220	33	-	100	-	1323	1699
	e	32	210	32	120	-	-	-	32	-	32	-	110	32	220	610	150	33	-	110	-	874	739

* normal alkane denoted where chain length given as C_{14}, C_{15}, etc.

Table 6. Selected aromatic hydrocarbons extracted from Mytilus edulis tissue, Port Angeles harbor (P) and Dungeness Bay (D).

Sample	Concentration, ng/g dry tissue		
	Phenanthrene	Fluoranthene	Pyrene
P-1 a	130	1000	400
b	170	1400	590
P-2 a	86	1000	460
b	100	740	360
c	64	950	400
d	71	740	340
e	120	540	230
P-3 a	110	370	130
b	180	290	160
P-4 a	190	560	170
b	57	640	140
P-5 a	150	1100	140
b	170	1100	170
D-1 a	-	-	-
D-2 a	-	-	-
b	-	-	-
D-3 a	-	-	-
b	-	-	-
c	-	-	-
d	-	-	-

Table 7. Microgravimetric analysis of hydrocarbons extracted from *Thais lamellosa* tissue, Port Angeles harbor (P) and Dungeness Bay (D).

Sample	Silica Gel Chromatography Fraction (µg/g dry tissue) 1. (saturated)	2. (unsaturated)
P-1 a	480	210
b	22	180
P-2 a	85	270
D-1 a	18	230
b	12	300
c	20	250
d	46	470
D-2 a	1.5	690

Table 8. Alkanes extracted from Thais lamellosa, Port Angeles harbor (P) and Dungeness Bay (D).

Alkane* concentration, ng/g dry tissue

Sample		C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	Pristane	Phytane	Σodd-C	Σeven-C
P-1	a	28	50	28	150	66	82	26	-	26	35	25	58	47	110	520	60	-	25	1900	620	570	766
	b	14	51	28	130	33	-	-	-	-	23	20	-	26	100	240	50	33	-	1300	360	354	394
P-2	a	-	31	30	-	34	-	62	-	-	-	-	-	-	60	130	65	-	-	500	130	156	256
D-1	a	-	42	17	96	-	-	-	-	-	-	-	-	-	-	390	65	-	-	210	-	203	407
	b	-	34	-	76	-	-	-	-	-	-	-	-	-	-	280	43	-	-	250	-	153	280
	c	-	34	11	140	14	-	-	-	-	-	-	-	-	45	820	100	-	-	330	-	319	845
	d	-	50	-	97	-	-	-	100	-	31	42	65	53	160	310	140	-	74	350	-	717	405
D-2	a	-	16	-	24	-	-	-	26	-	-	-	-	-	-	180	64	-	-	150	-	130	180

* normal alkane denoted where chain length given as C_{14}, C_{15}, etc.

Table 9. Selected aromatic hydrocarbons extracted from Thais lamellosa tissue, Port Angeles harbor (P) and Dungeness Bay (D).

Sample		Concentration, ng/g dry tissue		
		Phenanthrene	Fluoranthene	Pyrene
P-1	a	160	210	200
	b	100	160	160
P-2	a	280	200	130
D-1	a	-	-	-
	b	-	-	-
	c	-	-	-
	d	-	-	-
D-2	a	-	-	-

Table 10. Reproducibility of replicate GC sample injections (N = 5).

Alkane	Rel. Std. Dev. %
C14	2.3
C15	4.4
C16	1.9
C17	2.5
C18	2.8
C19	3.4
C20	6.6
C21	6.0
C22	5.9
C23	8.3
C24	10.0
C25	12.4
C26	12.0
C27	16.9
C28	12.3
C29	14.1
C30	51
C31	38
Pristane	2.9
Phytane	3.6

Table 11. Unsaturated compounds identified in Peabody Creek sediment.

Compound	Number of isomers found
toluene	
xylene	3
pinene*	
C_3 - benzene	6
C_4 - benzene	10
C_5 - benzene	4+
naphthalene	
C_6 - benzene	1+
dichlorobenzene*	1
methylnaphthalene	2
C_2 - naphthalene	7
C_7 - benzene	
C_3 - naphthalene	14
fluorene	
C_4 - naphthalene	18
phenathrene	
anthracene	
methyl fluorene	2
methyl phenanthrene and/or	5
methyl anthracene	
C_2 - (phenanthrene and/or anthracene)	10
fluoranthene	
pyrene	
benzanthracene	
chrysene	
benzofluoranthene	1
benzpyrene	2
perylene	

* non-petrogenic

Table 12. Selected aromatics suggested to be reported in the baseline study

1. n-propylbenzene	10. methylphenanthrene
2. i-propylbenzene	11. fluoranthene
3. naphthalene	12. pyrene
4. 1-methylnaphthalene	13. chrysene
5. 2-methylnaphthalene	14. benz(a)anthracene
6. biphenyl	15. benzo(e)pyrene
7. dibenzothiophene	16. benzo(a)pyrene
8. phenanthrene	17. perylene
9. anthracene	

RECOMMENDATIONS

General

This pilot study has demonstrated that methodology exists to detect and measure a number of hydrocarbons in sediments, mussels (Mytilus edulis and Mytilus californianus), and a snail (Thais lamellosa). The use of this methodology in an area relatively polluted with oil (Port Angeles) and in a relatively unpolluted area (Dungeness Bay) has revealed substantial quantitative differences in these compounds. During the first year, major emphasis should be given to seasonal variations and broad geographical coverage with minor effort devoted to widening the list of compounds and trophic levels under study. Work should continue to emphasize analysis of sediments since this is where indications of the accumulation of petroleum contamination can be expected. Past and current problems with water column analyses preclude main reliance on water as a sample matrix but further study of it is warranted.

A number of parameters important in baseline studies are undefined. For example, the optimum interval for sampling for baseline studies has not been established; seasonal differences are unknown. Areas having the highest and lowest probable petrogenic contamination thus should be sampled more frequently (e.g., twice quarterly). If hydrocarbon levels in these areas fluctuate significantly, the program should be flexible enough to allow even more frequent sampling. If possible, compounds such as the cycloalkanes (naphthenes) and a larger number of aromatic compounds including heterocyclics, should be surveyed. Also, compounds which survive weathering and biodegradation or result from these processes should be included in the analyses. Although trophic levels were treated minimally in the pilot study (snails which feed on the mussels), this area of study could be expanded. Attention could be given to studying special situations such as times of physiological stress in biota (e.g., spawning cycles) and areas where microbiological sampling is planned.

First-Year Recommendations

As a result of the pilot study and in consultation with the workshop panels of experts, the following recommendations are made for the first year of a PETROLEUM HYDROCARBON BASELINE INVESTIGATION FOR NORTHERN PUGET SOUND AND STRAIT OF JUAN DE FUCA.

Sample environment. Sampling of the intertidal zone should be emphasized. The intertidal zone is a major point of contact between surface-borne oil pollution and marine biota and sediment through the action of wind, surf, and tides. The intertidal zone normally serves as a vital shelter for a multitude of juvenile and mature biota, some of which are particularly vulnerable to parts-per-billion (ppb) levels of certain petroleum hydrocarbons. Through various processes petroleum hydrocarbons are sorbed by intertidal sediments

and by biota exposed to them. These contaminants eventually can be purged from such intertidal substrates by complex, incompletely-understood processes. However, many hydrocarbons are retained in the environment weeks or months after introduction. A logistical advantage of the intertidal zone is its accessibility for reproducible, periodic sampling by land.

Target samples. Sampling of sediment and Mytilus sp. should be emphasized. Sedimentary beaches exist throughout the study region and Mytilus sp. is ubiquitous in the intertidal zone. Contamination of the intertidal zone by petroleum hydrocarbons will be reflected in these substrates. Both substrates are amenable to reproducible and periodic sampling and should be sampled simultaneously at the same site to allow correlation of results.

Site selection. Sampling areas should be evenly distributed throughout the region. Maps of suitable areas are given in Appendix B. Sampling sites should include representatives from zones presumed to be relatively uncontaminated. Such intertidal sites provide the lowest hydrocarbon baseline data and, therefore, allow the clearest early indication of a change due to incipient petroleum pollution. Appendix B covers a number of such prospective sites (a-h) throughout the region, which have been examined by road maps, marine charts, and by seaplane to establish their general suitability (e.g., access, beach type and extent). These sites comprise a geographical grid covering the region. Sampling areas also should include those believed to be contaminated (i-k, Appendix B).

Sampling areas should include a variety of sedimentary beach types. Beach types vary according to their slope, exposure to wave action, sediment grain size, and density and diversity of biota. All of these variables have been shown to affect the disposition of hydrocarbon contaminants in other areas. Sampling areas should coincide, where possible, with those of other related studies in the region. Each sampling area should contain both sample types (sediment and mussel) in close proximity to permit correlation of results.

Sampling. Samples should be collected quarterly at constant tidal elevations. Sediment grain size generally increases with increasing tidal elevation while biotic density declines generally below the zero-foot tide level. Because small sediment grains tend to retain petroleum contaminants and since knowledge of the interrelation of petroleum contamination in biota is desired, the range of 0 to +3 feet tidal elevation should be sampled.

Sediment sampling should be carried out according to a "systematic-stratified" scheme. Core samples (20-100) should be collected to a constant depth (e.g., 3 cm) at regular intervals along at least two different tidal elevations over a distance of 50 meters or greater and combined to make a single composite sample. A second composite sample should be collected according to the same procedure. This scheme should provide adequate statistical representation of the sample site within normal analytical variability.

Specimens of Mytilus sp. should not be collected from pilings. This study showed that these organisms absorb aromatic hydrocarbons from creosote treated pilings. Specimens of Mytilus sp. should be within a given size range (e.g., 2-4 cm length).

Field measurements and laboratory analyses. Field conditions at each sampling time should be described, including wave exposure, weather conditions, and air, water, and sediment temperature. Sediment samples should be described according to physical characteristics (color, layering, etc.). The location and substrate in which the mussels are found should be described. Each sampling location should be documented each sampling time by photography.

Sediment composites should be characterized according to grain size. This is necessary for comparing results between samples since the retention of hydrocarbons is related to grain size. Total organic carbon should be determined in sediment composites and total lipids in mussel composites. Analytical results should be reported in terms of sample dry and wet weight.

Samples should be extracted and analyzed for residual hydrocarbons according to the procedures developed in the pilot study (Appendix A). Analytical results should include: (a) microgravimetric determination of total extractables, (b) total saturated and unsaturated hydrocarbons from adsorption chromatography, and (c) gas chromatographic determination of n-alkanes from C_{14} to C_{31}, pristane, phytane, and specific aromatic compounds listed in Table 12. Compound identities should be verified by mass spectrometry.

Special projects. Additional studies to be considered include:

1. Continued sampling at the Port Angeles/Peabody Creek site to provide information on changes in hydrocarbon concentrations with time at a site where chronic input is believed to have been stopped.

2. Evaluation of water column sampling and analysis techniques.

3. Sampling of sediment and Mytilus at one Pacific Ocean intertidal site (south of Cape Flattery) as a comparison.

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APPENDIX A

ANALYTICAL PROCEDURES

Materials*

Materials contacting the sample were confined to glass, Teflon, metal or residue-free solvents and reagents. This includes the liners of caps and lids. All glassware was washed in hot laboratory detergent, dried, and rinsed in sequence with reagent grade acetone and methylene chloride solvents dispensed from previously cleaned Teflon wash bottles. Teflon and metal foil sheeting and metal implements were also rinsed sequentially with acetone and methylene chloride before use. Highest purity reagents such as hydrochloric acid, anhydrous sodium sulfate, coarse sand, sodium hydroxide, silica gel, and glass wool were extracted with methylene chloride before use. Solvents employed in this study were the highest purity obtainable from Burdick and Jackson Laboratories, Inc., or Mallinckrodt Chemical Works. They were employed without further purification because they gave no measurable residues in procedural blank analyses. Other items are listed as follows:

Teflon wash bottles, 500 ml
Laboratory scalpels
Homogenizer - Tekmar Tissumizer No. SDT-182EN or Virtis Model 23
Test tube racks - A. H. Thomas Co., Cat. No. 9266-N32
Centrifuge tubes, 40 ml, with screw caps - Corning Glass Works, Cat. No. 8122
Teflon cap liners - A. H. Thomas Co., Cat. No. 2390H
Centrifuge - International Equipment Company, Model C5
Glass bottles, 1 oz. with screw caps and Teflon liners
Concentrator tubes, 25 ml - Kontes Glass Co., No. K570050, size 2525
Reflux columns - Kontes Glass Co., Cat. No. K569351, or VWR 1 mm
Ebullators (boiling tubes) - Kontes Glass Co., Cat. No. K569351, or VWR 1 mm glass tubes, VWR Cat. No. 32829-020 (cut to ca. 2.5 cm length and flame sealed at one end in laboratory)
Tube heater, 6-tube - Kontes Glass Co., Cat. No. K720003
Tube heater control unit - Kontes Glass Co., Cat. No. 720001
Adsorption chromatography columns - Kontes Glass Co., Cat. No. 42028
Glass (Pyrex) wool - Corning Glass Works, No. 3950
Silica gel, 100-200 mesh - MCB Cat. No. SX0144-06
Copper, fine granular - Mallinckrodt, Cat. No. 4649
Sand, coarse, reagent grade
GC sample vials - Hewlett-Packard, Cat. No. 5080-8712

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* Reference to a company or product does not imply endorsement by the U. S. Department of Commerce to the exclusion of others that may be suitable.

GC Teflon lined vial caps - Hewlett-Packard Cat. No. 5080-8703
Vial capper - Hewlett-Packard Cat. No. 871-0979
Dish, aluminum, utility, 57 mm diameter
Ether peroxide test paper - EM Laboratories, Inc., Cat. No. 10061-9G
Sediment extraction glass bottle, 1 liter - Scientific Products Cat. No. B 7573-IL
Ball mill tumbler - Model 8-RA, Scott-Murray, 8511 Roosevelt Way NE, Seattle WA 98115
Automatic gas chromatograph - Hewlett-Packard Model 5840, dual FID
Automatic GC sampler - Hewlett-Packard Model 7671A
GC columns, 30 m L x 0.25 mm ID, wall coated, glass capillary (SE-30) - J & W Scientific, P. O. Box 216, Orangevale CA 95662
Gas Chromatograph/Mass Spectrometer and Data System, Dual EI/CI - Finnegan, Model 3200

Dry Weight Determination

Sediment. Thaw sediment and remove pebbles by spatula or sieve. Thoroughly mix by spatula. Add 10-20 g of the sediment to a tared aluminum dish. Weigh and record the weight of dish and sample. Cover the dish and sample loosely with aluminum foil. Dry the sample in an oven at 120°C for 24 hr, then remove and cool for 30 min in a dessicator. Reweigh and record dried weight. Calculate percent dry weight as:

$$\frac{\text{weight (final)} - \text{weight (tare)}}{\text{weight (initial)} - \text{weight (tare)}} \times 100$$

Tissue. Place ca. 3 g clean coarse sand and a glass spatula in an aluminum dish and dry overnight in a 120°C oven. Cool the dish in a dessicator for 30 min. Weigh and record as tare weight.

Weigh into the dish, to the nearest mg, 0.5 g of sample. Using the spatula, mix the sample thoroughly with the sand, taking care to avoid loss of sand granules. Dry the sample in a 120°C oven for 24 hr, then remove and cool in a dessicator for 30 min. Reweigh and record the dried weight. Calculate percent dry weight as:

$$\frac{\text{weight (final)} - \text{weight (tare)}}{\text{weight (initial)} - \text{weight (tare)}} \times 100$$

Tissue Extraction

Mussels. Pry open the shells with a clean spatula and separate the two halves by severing the adductor muscle. Scrape the tissue from the shell into a tared 100 ml beaker for compositing with other individuals.

Snails. Place specimens between several sheets of clean foil and crack the shells by striking them firmly with a hammer. Remove the shell fragments (with clean forceps or spatulas), peel off the foot and deposit the tissue in a tared 100 ml beaker. The remainder of the procedure is identical for both molluscs.

Transfer the tissue sample to a homogenizer tube and blend with the homogenizer at medium speed for at least 30 seconds. Return the tissue to the original pre-tared beaker and weigh to assure that the sample amount is sufficient for the procedure. Weigh 10 g (to nearest 0.1 g) of sample into a tared 40 ml screw-capped centrifuge tube. Add 6 ml of 4N sodium hydroxide to each sample and to one empty tube for a reagent blank. Cap each tube tightly with a Teflon-lined screw cap, shake vigorously for 1 min, and place each sample tube in an oven at 30°C for 18 hr (overnight). Cool the samples to room temperature and shake to check completeness of digestion. If well digested, add 15 ml of peroxide-free diethyl ether, recap tubes tightly, and shake vigorously for 1 min. Check the caps for tightness, then centrifuge the tubes at 3000 RPM for 10 min. If the upper ether phase is clear, transfer it with a Pasteur pipet to a 1 oz sample bottle equipped with a Teflon-lined screw cap. Avoid any carryover of the lower aqueous plase. If the

supernatant ether phase is not transparent, see note 1 below before proceeding. Add approximately 0.5 g of anhydrous sodium sulfate to each bottle without agitation or swirling. Repeat the extraction with 10 ml of ether and combine the extracts. Cap the bottles tightly, swirl briefly and allow to settle for 10-15 min. A persistent turbidity indicates the presence of residual water which must be removed by additional anhydrous sodium sulfate before proceeding (see note 2 below).

Transfer the dried ether extracts to a 25 ml concentrator tube, attach the reflux column, and add a micro-ebullator (boiling tube). Place the apparatus in the tube heater at 80°-85°C (see note 2). Shroud the apparatus with aluminum foil to enhance distillation. Concentrate the solution to 2 ml and remove concentrator tubes from the heater. Add 2 ml of hexane and a second micro-ebullator, and concentrate to 1.8 ml to completely remove the ether. If the extract is turbid or viscous, column flow will be restricted. Such a sample should be dissolved in methylene chloride and filtered through a short (1-2 cm) silica gel column with methylene chloride. A bed of silica gel on a small fritted-glass (coarse) Buchner funnel is suitable. The methylene chloride solvent in the eluate should then be concentrated and displaced by hexane. The sample is now ready for microgravimetry and silica gel chromatography.

Notes:

1. If the emulsion layer is small, remove clear ether layer and proceed to the second extraction. If the emulsion is extensive, add about 1 g anhydrous sodium sulfate to the mixture and shake and centrifuge as before. Transfer the clear supernatant ether phase to the 1 oz bottle and proceed with the second extraction.

2. Care must be taken to avoid bumping during evaporation to avoid loss of the sample. Incomplete removal of water is the principal cause of this bumping, as indicated by the turbidity noted earlier. During evaporation, residual water comes out of solution as a separate phase at the bottom of the concentrator tube. This phase plugs the ebullator and halts boiling, leading to overheating and bumping. The remedy is to dry over more anhydrous sodium sulfate for a longer time and to carefully transfer the ether phase, avoiding any aqueous phase. In extreme cases, a double-ended ebullator may be used.

Sediment Extraction

Accurately weigh 100 g of pebble-free sediment into a 1 liter bottle fitted with a Teflon-lined screw cap. Add 50 ml of 0.1 N hydrochloric acid and 100 ml of ethanol-free, peroxide-free diethyl ether to the sample in the 1 liter bottle. Roll the sample on the ball-mill tumbler for 18 hr (overnight). Decant the supernatant ether phase through a glass-wool plug in a powder funnel into a 500 ml erlenmeyer flask. Add another 100 ml of ether to the slurry and roll again for 1 hour. Decant the ether extract into the same flask. Concentrate the extracts to ca. 15 ml by swirling the 500 ml erlenmeyer in a pan of warm (tap) water in a well-ventilated hood. Transfer

the ether extract with washing to a 25 ml concentrator tube, add an ebullator and a reflux column. Concentrate to 2 ml, then add 2 ml of hexane and a second ebullator and concentrate to 1.8 ml to remove ether. The extract is now ready for microgravimetry and silica gel chromatography.

Silica Gel Chromatography

Column Preparation. Prepare columns immediately prior to use. Fill a column to the flare in the reservoir with methylene chloride. Push a 0.5 cm glass-wool plug to the bottom of the column with a glass rod. Measure 15 ml (7 g) of 100-200 mesh silica gel (activated at 150°C for 24 hr, then cooled in a dessicator) into a 25 ml graduated cylinder and transfer to a 250 ml erlenmeyer flask. Add 25 ml of methylene chloride and swirl vigorously to make a slurry. Place a long-stem funnel into the column such that the tip rests off-center on the bottom of the reservoir just below the surface of the methylene chloride.

Quickly pour the slurry into the funnel and wash the residual slurry into the funnel with methylene chloride from a Teflon wash bottle. The adsorbent particles should quickly settle to the bottom of the column with little turbulence at the settling front. When the settling front extends upward about 1 cm from the glass-wool plug, slowly open the stopcock to a flow of 1-2 drops per second. Collect the eluate in an erlenmeyer flask to minimize solvent vapor escape. Swirl the column reservoir gently to wash the particles into the column. When the settling front reaches the top of the suspended particles, open the stopcock all the way to complete the settling. Add about a 1 cm layer of clean sand through a funnel to the top of the gel, followed by an equal amount of anhydrous sodium sulfate.

When the methlene chloride surface is just above the top of the column, add a ml of petroleum ether with a Pasteur pipet and allow to drain. When the liquid level again almost reaches the column top, add 40 ml of petroleum ether and continue to elute. Close the stopcock when the solvent meniscus almost reaches the top of the column. Discard the rinse elutes. Cover the column with aluminum foil until use.

Sample Chromatography. The sample extract should be in 1-2 ml of hexane in the concentrator tube. Crush the ebullator with a glass rod and rinse the rod with a small amount of petroleum ether. Carefully transfer the extract solution with a Pasteur pipet to the top of the column and elute. Never allow the liquid meniscus to go below the upper surface since air will be entrapped, which will disrupt the column. Rinse the concentrator tube with 0.5 ml of petroleum ether and add to the column. Open the stopcock and collect the eluate in a clean 25 ml concentrator tube. When the meniscus just reaches the column top, carefully add 15 ml of petroleum ether. Care must be exercised not to disturb the upper surface of the column during each addition. When the meniscus again just reaches the sand, add 3 ml of 20% (V/V) methylene chloride in petroleum ether. Elute solvent at 2-4 ml/min to separate the saturated from the unsaturated hydrocarbons. When 18 ml has eluted into the concentrator tube receiver, replace it with a second tube. This 18 ml eluate, referred to as fraction 1, contains the saturated

hydrocarbons. As the meniscus again just reaches the top, add 25 ml of 40% (V/V) methylene chloride in petroleum ether. This eluate, fraction 2, will contain the unsaturated and aromatic hydrocarbons. A transparent extract, when applied to the column, will elute in less than 30 minutes.

Sediment Desulfurization. Silica gel fractions of sediment extract are treated with activated, fine granular copper to remove elemental sulfur. Prior to use, activate the copper with concentrated hydrochloric acid (HCl). Rinse the activated copper five times with acetone to remove the HCl and then five times with petroleum ether to remove the acetone. Activated copper should be prepared fresh daily and stored under petroleum ether until used. Activated copper should not be washed with water or heated. To remove elemental sulfur from the sample, place the eluate (not more than 1 ml in volume) in a 40 ml conical centrifuge tube and add about 0.5 ml of activated copper. Stir for 2 minutes on a vortex mixer. Centrifuge to settle any sulfide particles in the mixture. Transfer the sample with a Pasteur pipet to a clean concentrator tube. Rinse the copper once with 1 ml of petroleum ether and combine the rinse with the eluate sample. Reconcentrate the sample to a 0.5 ml and continue to microgravimetry and GC analysis.

Microgravimetric Determinations

The first and second silica gel fractions are weighed on a Cahn microbalance. In an efficient hood, transfer 25 µl from a known volume of eluate (or extract) onto the balance pan and allow the solvent to evaporate. Record the weight and normalize the value to µg/g dry weight of sample.

Gas Chromatography (GC)

GC Sample Preparation. Attach the reflux column to the concentrator tube containing the eluate from silica gel chromatography. Evaporate the solvent in the heater block as previously described. After concentrating to 0.5 ml, remove from heat. Add 1.0 ml of internal standard solution (4 ng/µl hexamethylbenzene in carbon disulfide) and concentrate to 0.5 ml. If necessary, adjust final volume to 0.5 ml with carbon disulfide. Transfer the samples to the GC vials and crimp on the Teflon-lined septum caps. Replace the cap each time it is pierced by a syringe to avoid evaporative losses.

GC Apparatus and Modifications. GC analysis is performed on a microprocessor-controlled gas chromatograph (Hewlett-Packard model 5840A) equipped with: an automatic sample injector (model 7671A); a wall-coated, open tubular (WCOT) glass capillary column (20-30 m length, 0.25 mm inside diameter); and a hydrogen flame-ionization detector (FID).

The GC sample injection port is modified to split the carrier gas as shown in Figure 1. Inlet carrier gas (helium) pressure is adjusted to provide 2 ml/min flow through the column at 60°C, as determined on a bubble flow-meter. By adjusting the needle valve to allow 20 ml/min bypass flow, a split ratio of 10:1 is obtained. Although 90% of the injected sample is sacrificed, the inlet system is rapidly purged of injected solvent and sample. This

maintains sharp solvent and sample peaks. This inlet system (Fig. 1) features low dead volume and a glass inlet liner that is readily removable for cleaning. The inlet end of the glass capillary column must be positioned inside the glass liner near the location of the inserted sampling needle tip to gain best sample transfer to the column with the least GC peak broadening. A charcoal trap absorbs compounds from the vented split stream which avoids contaminating the needle valve.

Because of the low, carrier gas flow through capillary GC columns, it is necessary to add make-up gas at the FID (Fig. 1). The flame jet has been flared to allow the GC column outlet end to be inserted about 2 cm into the jet. This effectively eliminates any potential dead volume effects with the make-up gas (30 ml/min) plus hydrogen (24 ml/min) rapidly sweeping eluted compounds directly into the flame.

GC Sample Analysis. Analysis is carried out according to conditions listed in Table 1. GC samples in crimp-sealed, septum-capped vials are loaded into the automatic sampler. Then the desired operating conditions (Table 1) are programmed into the microprocessor memory. A sample volume of 2 µl are injected per analysis with the column temperature held at 60°C. After 10 min, the column temperature is programmed at 2° or 4°C/min to 250°C and held for 30 minutes. Depending on the program rate, the compounds of interest are eluted in 1½ to 2½ hours. Separated compounds are detected by the FID as they emerge from the GC column. The gas chromatogram is constructed by the microprocessor, which prints compound retention times alongside each peak.

Peak areas are automatically computed using "valley to valley" mode baseline correction. Areas are printed in tabular form at the end of the GC run according to retention times. The quantities of compounds represented by the peak areas are also computed automatically by ratio of the individual peak areas to the area of the known amount of internal standard peak. If reference samples are available for compounds of interest, relative response factors for these compounds with respect to the internal standard should be determined experimentally under identical conditions.

Gas Chromatography/Mass Spectrometry (GC/MS). The identity and relative abundance of compounds detected and measured by GC are periodically confirmed by GC/MS analysis. A capillary column similar to that used in GC analysis is employed in conjunction with a Grob sample inlet system. Effluent from the GC column is fed directly into ion source. Table 2 lists analysis conditions. A sample of 1-2 µl is injected into the GC/MS while the ion source filament and electron multiplier voltage are turned off. Passage of the solvent peak from GC to MS is noted on the instrument high vacuum gage as a transient rise and fall in pressure. After this, the source filament and multiplier voltage are restored to normal settings and data acquisition by the computers is initiated for mass scans every 2 sec. The GC column is subjected to virtually the same analytical parameters for the GC/MS confirmation run as in the GC detection and measurement run. At the end of the run, the chromatogram is reconstructed (RGC) from the total ion current of each individual scan. Specific ion chromatograms featuring ion abundancies of ions characteristic of a particular molecular configuration may also be produced. Primarily,

Table A-1

Gas Chromatography Conditions

Column type		Column:	30 m x 0.25 mm ID wall-coated glass capillary
		Liquid phase:	SE-30 GC (dimethyl siloxane polymer)
		Film thickness:	$4\text{-}5 \times 10^{-4}$ mm
Gases	Inlet	Carrier gas:	He
		Split ratio:	10:1 (bypass:column)
		Column flow:	2 ml/min
		Bypass flow:	20 ml/min
	Detector	Makeup (N_2)	30 ml/min
		Air	240 ml/min
		Hydrogen	24 ml/min
Temperatures		Initial Temp:	60°C
		Program delay:	10 min
		Program rate:	2 or 4°C/min
		Final temp:	250°C
		Injector:	250°C
		Detector:	300°C

compounds shown to be present in the GC/MS chromatogram are identified by comparing their mass spectrum (background subtracted) with standard reference tables of mass spectra or laboratory spectra of reference compounds.

Table A-2

GC/MS Analysis Parameters

GC: Same as Table A-1, except no make-up gas

GC/MS interface temp.: 250°

MS:

Filament emission:	500 μA
Electron multiplier voltage:	1600 V
Electron energy:	70 eV

Data acquisition:

Mass range:	80-280 (aromatic samples) 50-300 (alkane samples)
Integration time:	6 msec/scan
Scan time:	2 sec

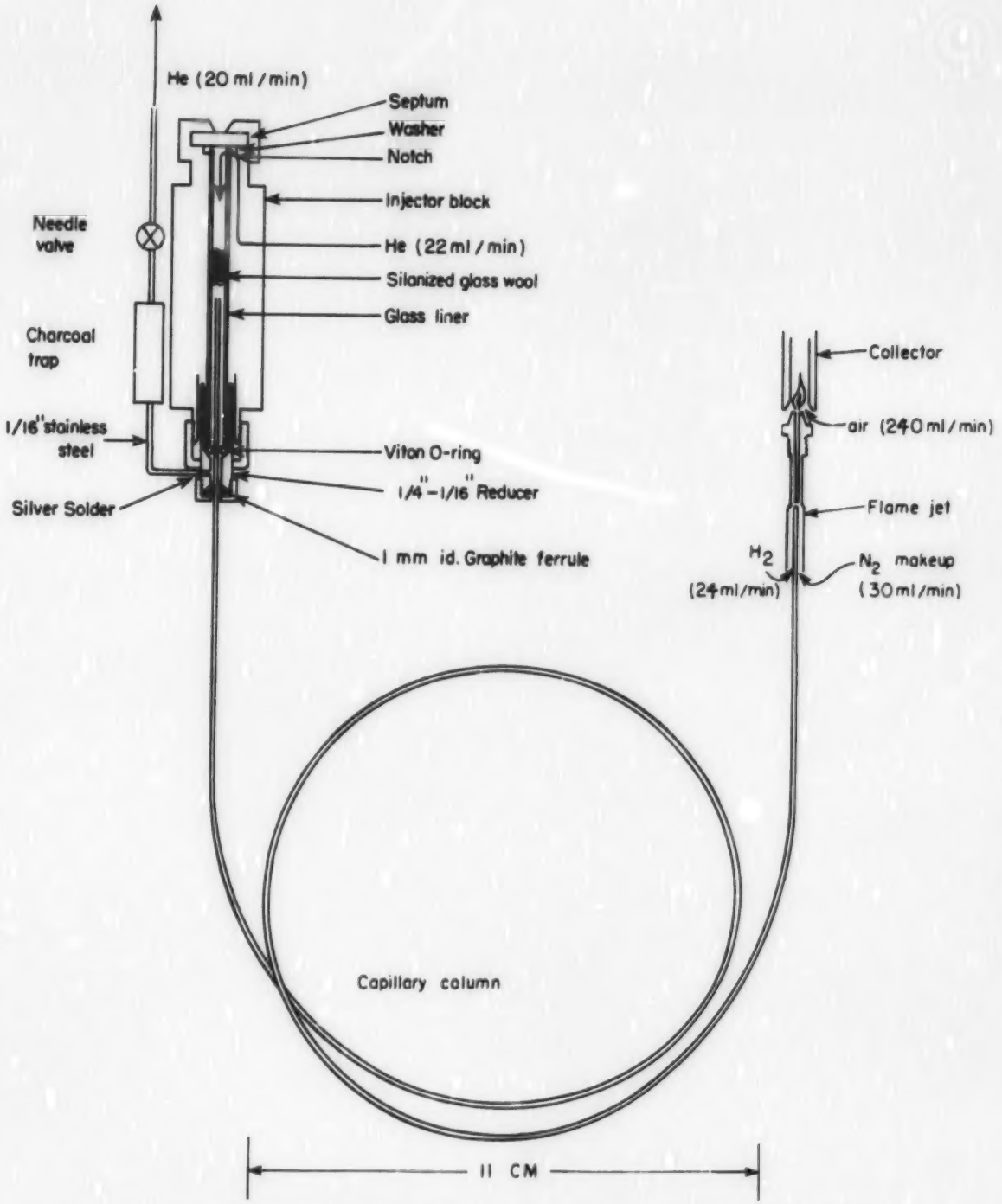


Figure A-1. Schematic details of the GC sample train:injector, column and detector.

APPENDIX B

DETAILED MAPS OF RECOMMENDED SAMPLING AREAS

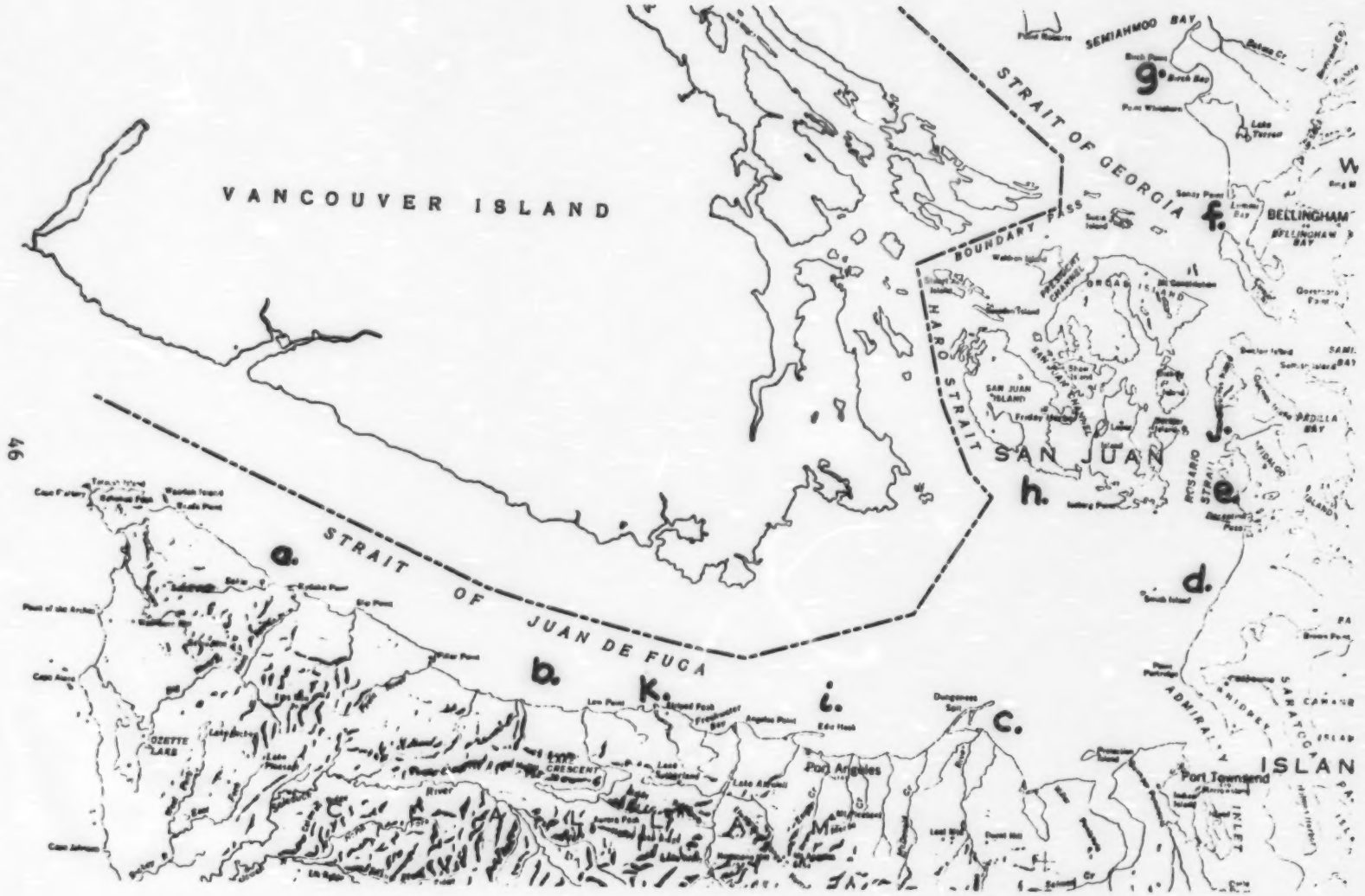
(Intertidal sampling areas found suitable from aerial survey, August 9, 1976)

Relatively unpolluted

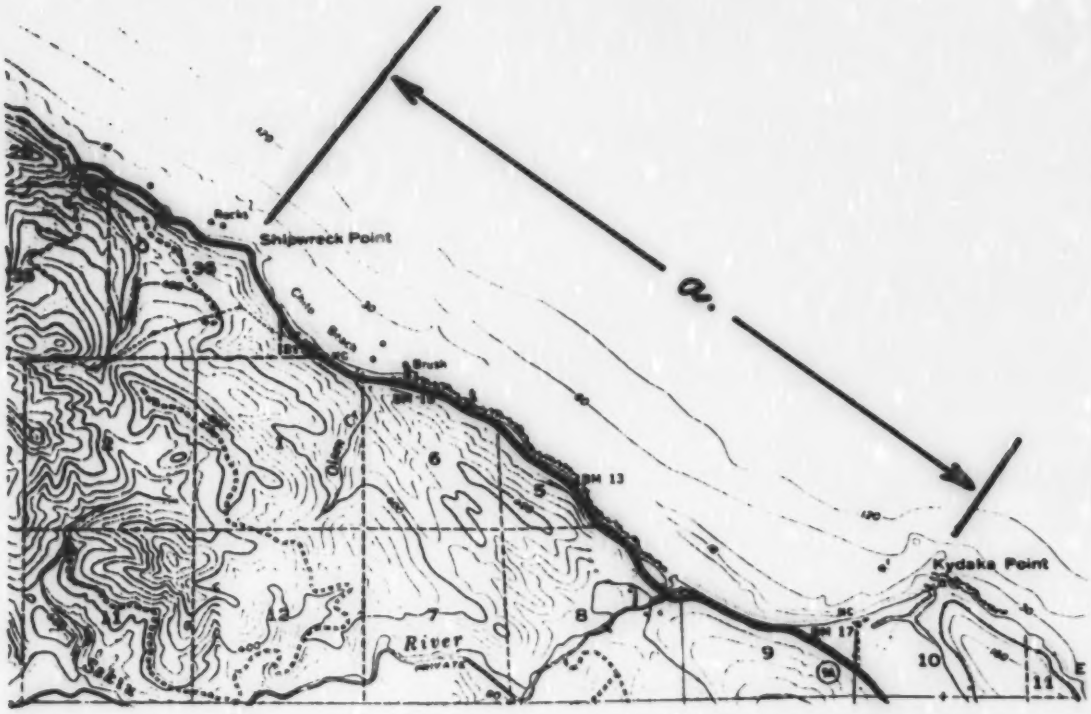
Code	Area	Site	Page
a.	Western Strait	Chito Beach or Kydaka Point	B-3
b.	Central Strait	Pillar Point, Agate Bay or Freshwater Bay	B-3,4
c.	Eastern Strait	Dungeness Bay or Jamestown	B-4
d.	Whidbey Island	One of several beaches from Partridge Point north to the Naval Air Station	B-5
e.	Fidalgo Island	Telegraph Bight or Langley Bay	B-5
f.	West Lummi Bay	Sandy Point	B-6
g.	Birch Bay	Birch Point	B-6
h.	San Juan Island	Cattle Point or False Bay	B-7

Relatively polluted

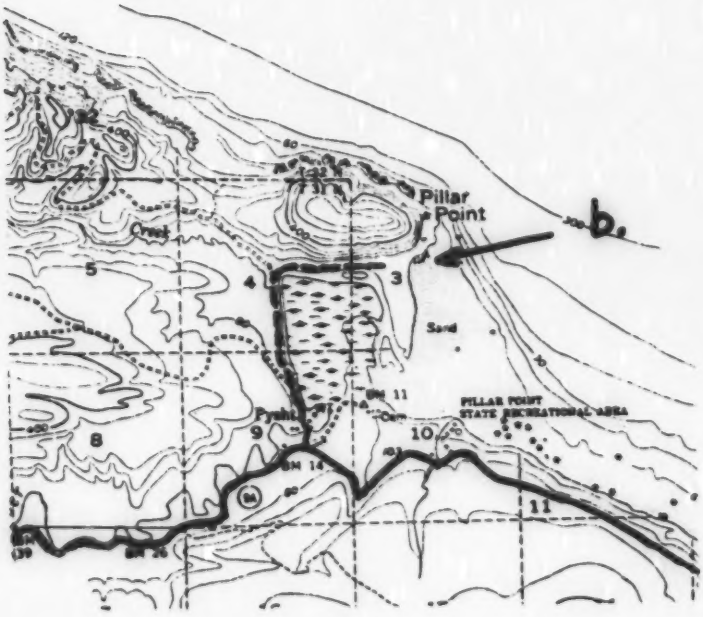
i.	Port Angeles	Peabody Creek or Morse Creek	B-8
j.	Fidalgo Island	Shannon Point or Green Point	B-8
k.	Central Strait	Crescent Bay	B-4



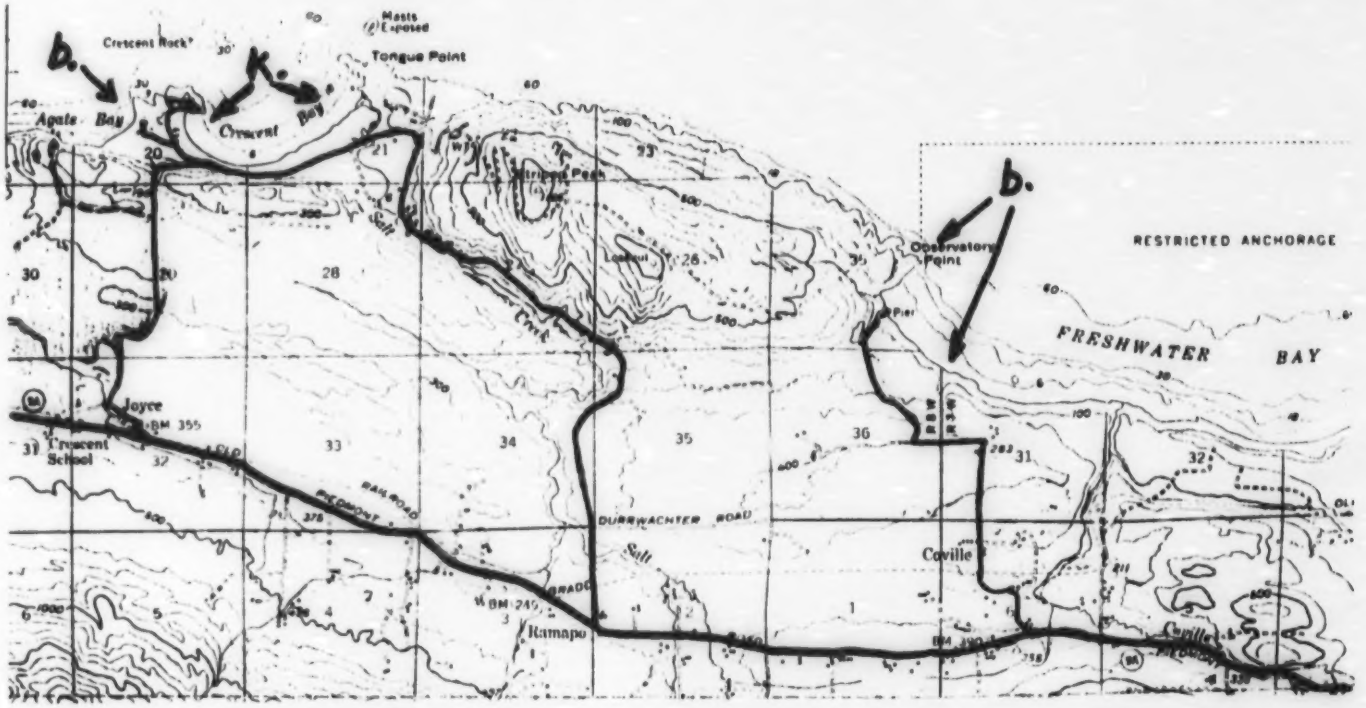
Northern Puget Sound and Strait of Juan de Fuca



a. Western Strait: Chito Beach or Kydaka Point

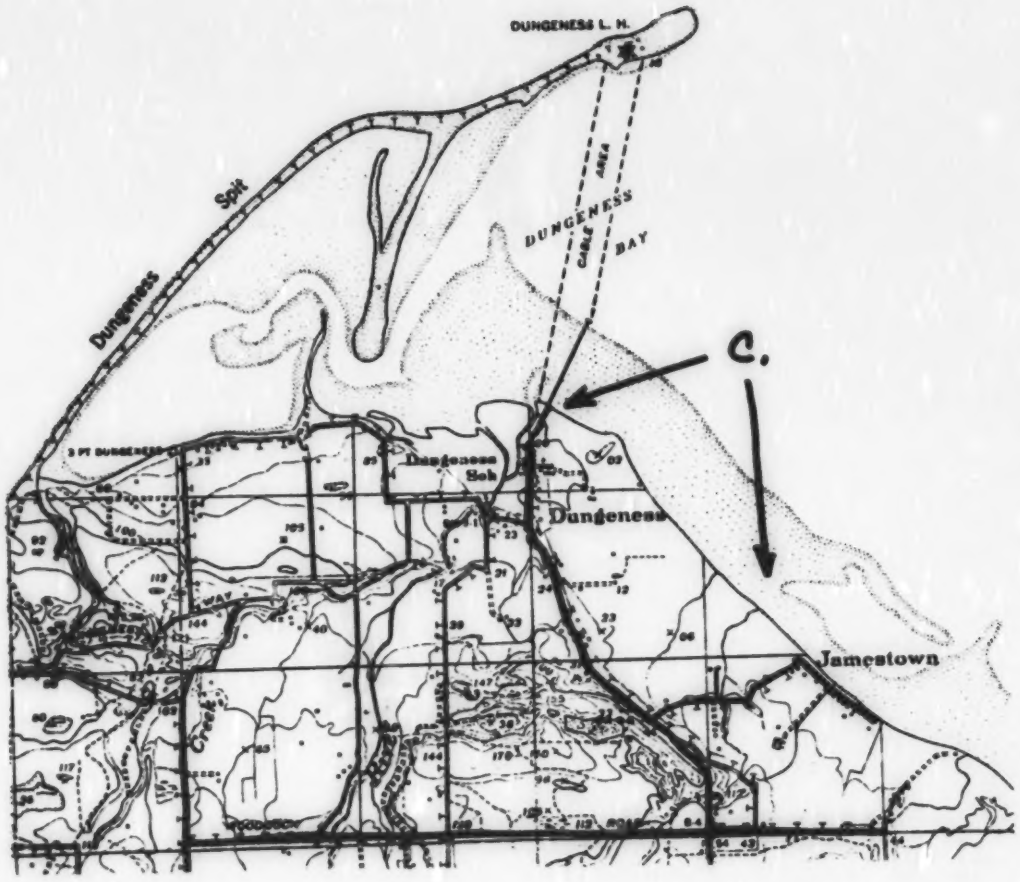


b. Central Strait: Pillar Point

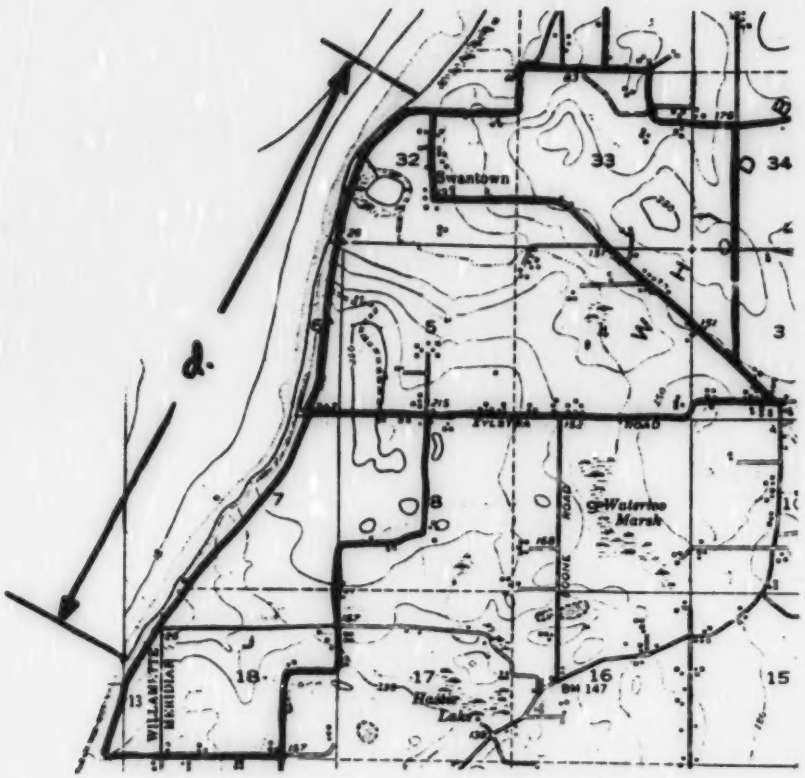


b. Central Strait: Agate Bay or Freshwater Bay

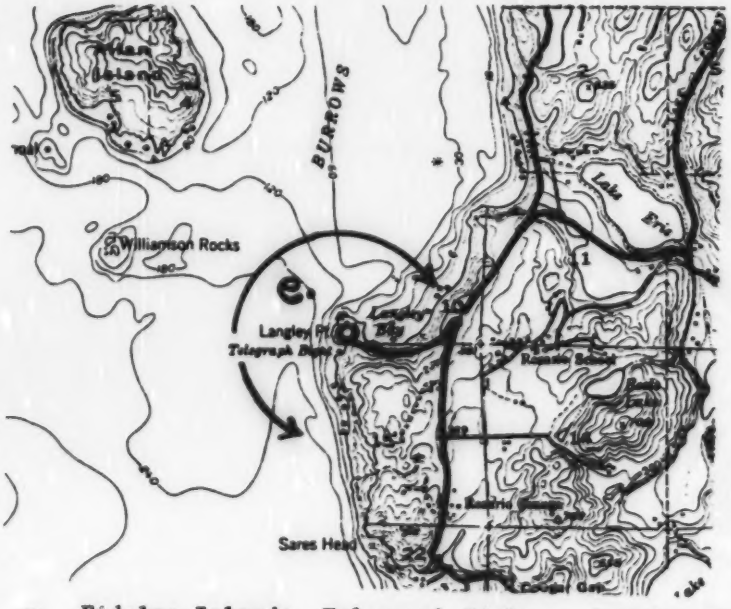
k. Central Strait: Crescent Bay



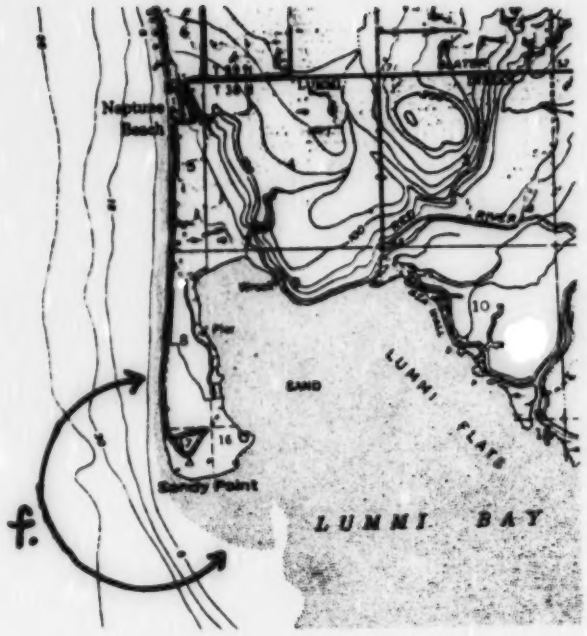
c. Eastern Strait: Dungeness Bay or Jamestown



d. Whidbey Island

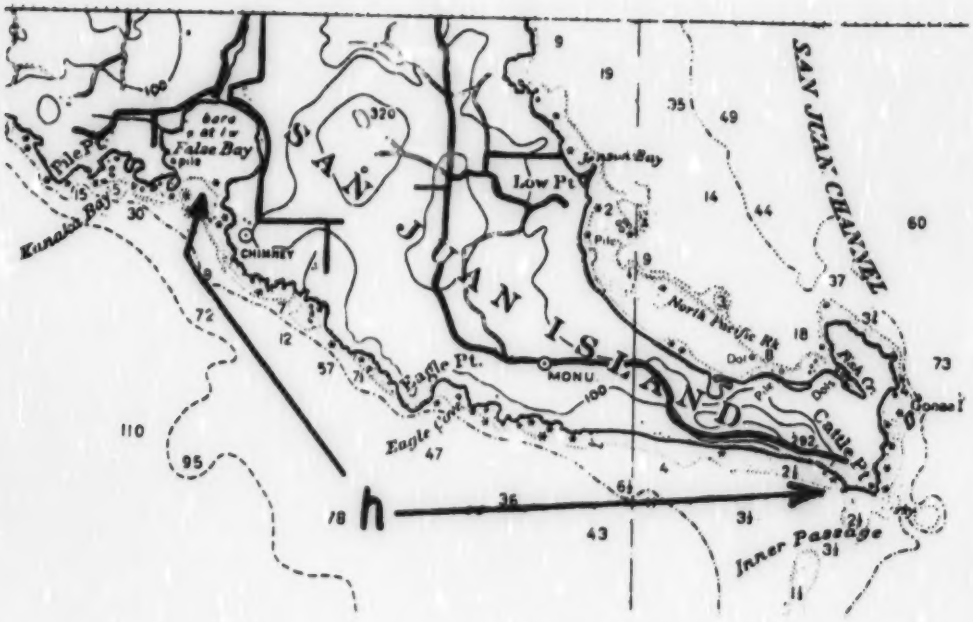


e. Fidalgo Island: Telegraph Bight or Langley Bay

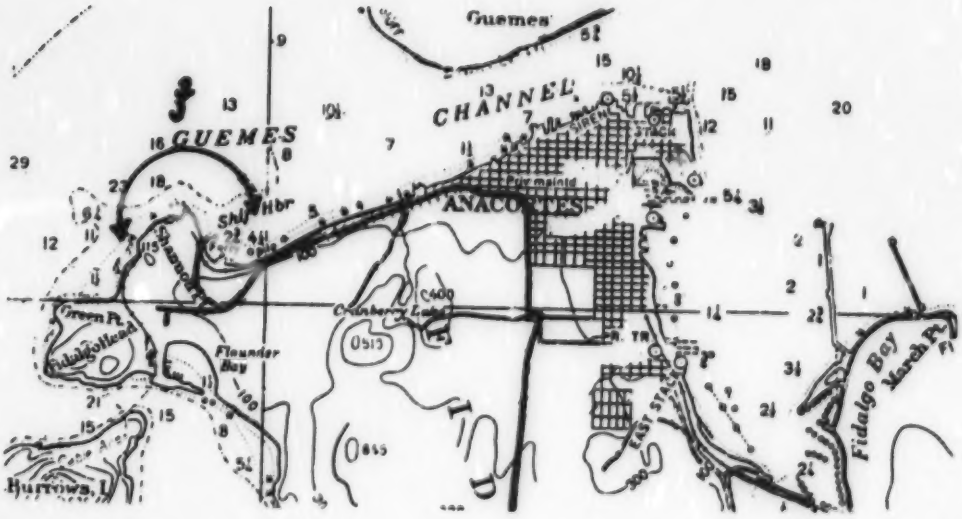


f. West Lummi Bay: Sandy Point

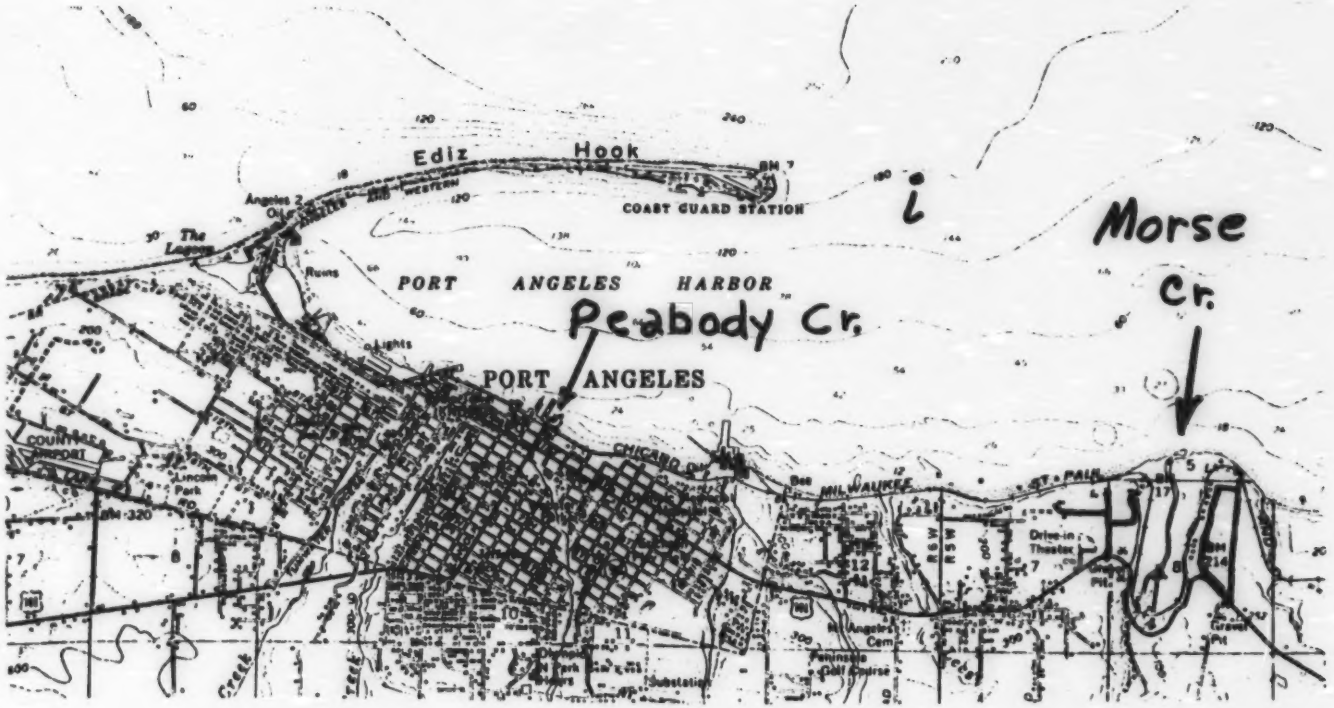
g. Birch Bay: Birch Point



h. San Juan Island: Cattle Point or False Bay



j. Fidalgo Island: Shannon Point or Green Point



i. Port Angeles: Peabody Creek or Morse Creek

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

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3. RECIPIENT'S ACCESSION NO. PB274591	
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15. SUPPLEMENTARY NOTES
This project is part of the EPA-planned and coordinated Federal Interagency Energy/Environment R&D Program.

16. ABSTRACT
Hydrocarbon baseline data are needed to assess the potential impact of oil contamination from increased tanker traffic in the Strait of Juan de Fuca . Initial studies were directed to intertidal sediments, mussels and snails from two physically similar areas: Port Angeles, WA and Dungeness Bay, WA. Latest analytical techniques of solvent/slurry extraction, silica gel chromatography, and glass capillary gas chromatography were adapted for analyzing large numbers of samples. The methods apply to alkanes in the C_{14} - C_{32} range, or to aromatics from substituted benzenes through benzpyrenes. Individual hydrocarbons were determined within 20% standard error at the 10 ng/g level.

Lowest hydrocarbon levels were found at Dungeness Bay, a relatively pristine area. The profile of the alkanes suggests that the Dungeness hydrocarbons arise biogenically. Hydrocarbon levels were substantially greater at Port Angeles harbor, especially adjacent to Peabody Creek. The even distribution of C_{14} - C_{22} n-alkanes from creekside sediment is consistent with known fuel oil seepage upstream. Above C_{22}, the n-alkane pattern appears more biogenic, although the levels are high. Significant divergences between the two areas were found in the phenanthrene fluoranthene, and pyrene levels. Seventeen aromatic hydrocarbons are proposed for baseline monitoring.

17. (Circle One or More) KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS			b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field Group
Ecology (Environments) Earth Atmosphere Environmental Engineering Geography Other:	Hydrology, Limnology Biochemistry Earth Hydrosphere Combustion Refining	Energy Conversion Physical Chemistry Materials Handling Inorganic Chemistry (Organic Chemistry) Chemical Engineering		04 06 07 08 (13) 13 (14) 15 14B

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